MODIFYING AQUEOUS HUMOR DYNAMICS

FOR THE TREATMENT OF GLAUCOMA

by

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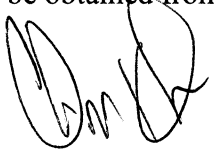
SIGNED: 

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ABSTRACT

Glaucoma is one of the leading causes of irreversible blindness in the world, and the leading cause in many developing countries. It is a progressive loss of optic nerve tissue typically caused by increased intraocular pressure and treated by lowering the pressure. In developing nations where the disease is most common medications are not affordable and surgery is expensive and unstable. A new surgical approach and drainage device material that could lead to a cost effective, permanent solution were studied. A tube connected to an e-PTFE reservoir shunts fluid from inside the eye to the subconjunctival reservoir. E-PTFE was chosen because it is biocompatible and porous, while promoting angiogenesis and lymphangiogenesis on its surface. The surrounding vessels can carry the fluid back into the systemic circulation. A tangential surgical approach was evaluated because it could spare and protect lymphatic vessels. Recent studies have shown that lymphatics are important in aqueous outflow. Preliminary results showed that the e-PTFE device could sufficiently lower IOP and that the lymphatics are potentially involved in aqueous outflow after surgery. These results indicate the importance of further evaluating a new surgical approach that addresses the role of lymphatics in fluid outflow from a glaucoma drainage device.

1. INTRODUCTION

1.1. What is Glaucoma?

Glaucoma is an affliction of the eye that results in the slow progressive degeneration of the retinal ganglion cells (RGCs) and optic nerve axons [Gupta]. It is the leading cause of irreversible blindness in many areas of the world, with no permanent treatment currently available. The main risk factor for glaucoma is elevated intraocular pressure (IOP), but there are other pathways and risk factors associated with glaucoma. Therefore, the treatment strategies for glaucoma typically attempt to lower the intraocular pressure. In normal eyes, the aqueous humor helps maintain the pressure necessary for the eye to keep its shape, while in many glaucoma patients the IOP becomes too high when the aqueous humor outflow mechanism malfunctions.

Aqueous humor in the eye is produced in the ciliary body and then flows into the anterior chamber to provide nutrients to different cells, including those of the cornea and lens. The aqueous humor then exits through the conventional trabecular meshwork and Schlemm's canal outflow pathway or the unconventional uveoscleral outflow pathway, which is thought to account for 10-15% of outflow in the eye (see Figure A.1 for the structure of the eye, and Appendix A for more on the types and prevalence of glaucoma) [Johnson 2006]. Other factors potentially involved in the development of glaucoma include high glutamate levels, variations in nitric oxide metabolism, and cellular damage from reactive oxygen species [Gupta]. Therefore, other therapies that attempt to increase blood flow, protect the neuronal cells in the retina, and modify gene expression in the eye should also be considered for the prevention of glaucoma [Vetrugno].

1.2. Why is Glaucoma Treatment to Lower IOP Important?

When left untreated, the damage to the RGCs and the nerve axons is irreparable and the resulting blindness is incurable. As the second cause of blindness and leading cause of irreversible blindness in the world, glaucoma has caused complete blindness in over 6 million people [Vetrugno]. Worldwide, almost 67 million people suffer from the disease, a number that is expected to rise to around 80 million by 2020 [Gupta]. Despite this, many people who are afflicted with glaucoma are not able to receive the proper care to prevent visual field loss. In the United States an astounding 27% of Medicare beneficiaries that are diagnosed with primary open angle glaucoma do not receive therapy in any given year [Stein]. The percent of patients who are diagnosed but do not receive treatment is greater among residents of metropolitan areas, Asians and Hispanics, making it a socioeconomic issue [Stein].

Additionally, in third world countries very few people who have glaucoma are actually treated, in large part due to the costs associated with the medications. Cost is a big issue with glaucoma, and it is estimated that the direct costs and lost of productivity of the illness costs a total of $35 billion per year in the United States [Fiscella]. Accurate diagnosis of glaucoma poses an issue as well since the RGCs can sustain damage prior to the occurrence of notable symptoms, and current techniques such as the tonometry can provide misleading data [Vetrugno]. It is estimated that glaucoma remains undetected in 50% of the population prior to vision loss [Vetrugno]. There is still controversy on when to begin treatment for those patients with suspected early stage glaucoma.

Some studies show that early detection and subsequent treatment can improve patients' outcomes and prevent the death of RGCs. Researchers who support early treatment of glaucoma believe that prolonged elevation of IOP can initiate the different processes involved in the destruction of the retinal ganglion cells [Schwarzt]. Once an elevated IOP is detected there are several factors considered in whether the patient is treated immediately or just observed for further progression, such as: "social-economic impact of a long-term treatment, likelihood that the treatment is useful for the patient, patient's health status, life expectancy, and patient's relative risk of developing glaucoma" [Vetrugno]. The prevalence of both untreated and undiagnosed glaucoma is a major problem, and it adversely affects a large number of people. Therefore, the main goal of physicians should be to look for a permanent solution to glaucoma while making current therapies accessible to more people in order to decrease visual loss from the disease.

1.3 How is Glaucoma Treated?

Typical methods used to treat glaucoma include pharmaceutical agents, laser treatments, or trabeculectomy surgery. Aqueous drainage devices, or glaucoma shunts, are currently only used as a secondary method of treatment because of the large number of complications and high failure rates [Boswell]. Even though pharmaceutical treatments are currently very common, a more permanent surgical solution would be preferred because it could improve the quality of life of glaucoma patients. Glaucoma drainage devices are the focus of this study because recent studies have shown their

promise for improved treatment of glaucoma in the future. In particular, the e-PTFE glaucoma drainage device has advantageous properties that make it a prime candidate to create a long-lasting aqueous outflow pathway.

In this review, the pharmacological treatments for glaucoma will first be introduced briefly. Then, the promising new e-PTFE shunt along with preliminary studies completed on the device will be discussed. After the e-PTFE implant is introduced, other novel glaucoma shunts will be reviewed in detail. An *in vivo* rabbit study performed to explore a new surgical technique while ascertaining the importance of lymphatic drainage is then presented. The traditional trabeculectomy surgery along with some newer surgical techniques that could potentially replace conventional methods are expounded upon after the surgical method of this study. The discussion of surgical glaucoma treatments is followed by a review of numerous imaging methods that could visualize aqueous humor outflow and the corresponding imaging studies.

1.4. Issues with Glaucoma Medications

Drug therapy is currently the most common method used to treat glaucoma in the United States, but there are several issues associated with the use of glaucoma medications. In many chronic diseases adherence is estimated to be 75% at best, and the population mainly affected by glaucoma (i.e. the elderly) face even more barriers to proper adherence [Tsai 2009, Stein]. Glaucoma patients have more challenges, including hearing difficulties, impaired vision, impaired cognitive abilities, decreased health literacy, reliance on others to apply medication, and limited financial resources. Risk

factors for poor adherence include complicated dosing regimen, patient-specific factors, provider factors, and situational factors. Safety precautions are often not observed by many patients when administering their medication, with only a third of glaucoma patients washing their hands prior to application and only a sixth using a mirror during application [Tsai 2009].

Patient education is essential in preventing both poor adherence to treatment plans and poor safety habits. Unfortunately for patients with severe glaucoma, the goal of a 40-50% reduction in IOP is difficult to reach with many of the marketed medications and therefore a combination of therapies or drugs with different modes of action often needs to be considered. In addition, there are many side effects that patients can experience with the use of these drugs and they can be inaccessible, especially for people in third world countries.

1.5. Types of Glaucoma Medications

There are several main classes of glaucoma medications, including beta blockers, prostaglandin analogs, adrenergic agents (including alpha agonists), carbonic anhydrase inhibitors, cholinergics, hyperosmotic agents, and investigational medications (glaucoma medications are discussed in more detail in Appendix B). Novel medications have offered improved efficacy and safety to glaucoma patients, but the search continues for a more permanent solution to glaucoma. An e-PTFE device that was explored in this study for use in glaucoma drainage surgery will be discussed in the next section.

2. E-PTFE GLAUCOMA DRAINAGE DEVICE

2.1. Advantages and Disadvantages of Drainage Devices

In developed countries, when conventional or laser surgery is not enough to lower the patient's IOP a device that acts as an artificial drainage pathway can be implanted to increase aqueous humor outflow. "Traditionally, glaucoma drainage implants have been reserved for patients with risk for failure with standard filtering surgery" [Nguyen]. Though in the past surgeons typically only used glaucoma drainage devices when necessary or in complicated cases, there is a growing trend towards the use of shunts for primary treatment as new evidence of their efficacy is continually being shown. Overall opinion is changing in favor of using shunts. Recently, a large experiment called the Tube Versus Trabeculectomy study compared the conventional trabeculectomy to the implantation of a shunting device and showed the benefits of using a drainage device [Gedde, Nguyen]. "After 1 year of follow-up, the study concluded that nonvalved tube surgery was more likely to maintain IOP control and avoid persistent hypotony or reoperation for glaucoma than trabeculectomy with mitomycin C," but "there was less need for medical management following trabeculectomy with mitomycin C" [Sarkisian].

Various complications can result with the surgical implantation of glaucoma drainage devices, including hypotony, diplopia, proptosis, tube erosion, failure, endophthalmitis, and visual loss. The most persistent complications associated with glaucoma shunts but not with conventional surgery are those related to cornea, including corneal endothelial loss or even corneal decompensation [Hau]. In many cases glaucoma drainage devices are not made from a biocompatible material, so the body sequesters the

implant with a fibrous response and surrounds it with a fibrous capsule. This fibrous capsule does not allow diffusion or passage of the aqueous fluid, and the shunts fail. Though tube blockage from blood, fibrous membranes, vitreous, inflammatory debris, or iris tissue is a cause of failure, inadequate capsular diffusion more commonly the reason why some shunts fail [Burchfield]. The e-PTFE shunt was designed to address the issues of improper immune response and low capsular diffusion.

2.2. Why e-PTFE?

In response to the clinical challenges that glaucoma poses a valve/shunt using a porous polymer, expanded polytetrafluoroethylene (e-PTFE) has been proposed (see Figure 2.1). It is thought that fluid flows from the reservoir and out through the venous channels surrounding the valve to lower the IOP. Through several studies e-PTFE has been shown to be very inert in the body, non-immunogenic, biodurable, and a material that when denucleated of trapped air in the appropriately sized pores will promote vascular growth [Schwartz]. Experiments have shown that e-PTFE denucleated prior to implantation has improved tissue incorporation, decreased thrombogenicity, and increased neovascularization when compared to conventional drainage devices [Boswell]. Though results of previous clinical studies with the e-PTFE device were not markedly different from previous aqueous drainage devices, they showed a lowering of IOP and room for improvement [Kim 2003].

Additionally, recent experiments with porous e-PTFE membranes around previous valve designs showed an altered tissue response indicating less fibrous growth

and enhanced vascularization [Decroos]. "Several groups have hypothesized that increasing the number of vessels in the tissue surrounding the implant will lead to improved and prolonged device function" [Schwartz]. Neovascularization is thought to have a huge benefit because the new vessels can bypass the fibrous capsule surrounding the device. In glaucoma drainage devices, the new vessels could act as collector channels to help flush fluid away from the subconjunctival bleb, preventing the back pressure in the reservoir and allowing for more efficient aqueous outflow. Typically, aqueous drainage devices are not successful because of fibrous capsule growth around the drainage device inhibiting aqueous humor outflow. However, evidence shows that there are no advantages to using antifibrotic agents with the currently available shunts, which makes angiogenesis around the device even more important [Minckler]. The improved vascularization and decreased immune response seen near the e-PTFE device could translate into improved patient outcomes, and it is therefore a novel device that should be considered for use in future glaucoma treatment.

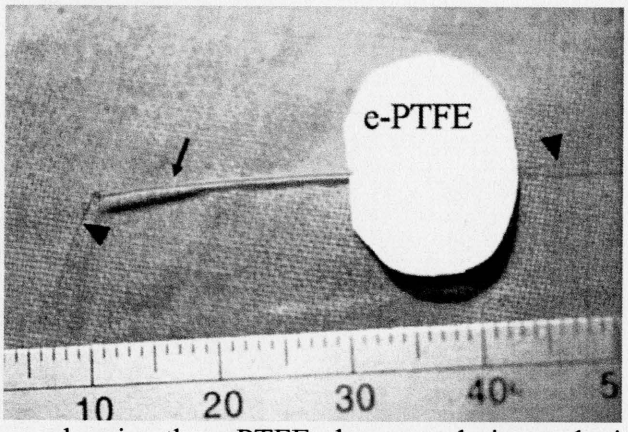

Figure 2.1 Image showing the e-PTFE glaucoma drainage device [Kim 2003]

2.3. Preliminary Flow Studies with the e-PTFE Device

Additional information about the e-PTFE glaucoma implant will be extremely helpful once it reaches the clinical trial stage in the United States. A preliminary experiment was completed to characterize several flow properties of the e-PTFE glaucoma shunt. To achieve this goal, multiple flow studies were performed on both the 1-sided and 2-sided e-PTFE glaucoma shunt. The flow rate was also measured at different pressures and at different bovine albumin protein concentrations. The flow rate studies were performed on two different e-PTFE 1-sided shunts (labeled as 1a and 1b) and two different e-PTFE 2-sided shunts (labeled as 2a and 2b) purchased from Impra, Inc. (Tempe, AZ). The setup for the experiment included a syringe connected to a tube, with either an 18 gauge needle to attach over the end of the e-PTFE shunt or a 28 gauge needle to attach inside the end of the e-PTFE shunt. Once the water or bovine albumin solution was poured in the syringe, it flowed down the tube because of the water pressure head, and the hydrostatic pressure was set to different values by changing the height of the syringe above the drainage device. The solution was allowed to flow through the device onto a petri dish, and the amount of liquid was weighed every minute to calculate the overall flow rate.

The results of the experiments conducted showed several interesting phenomena (Figure 2.2). First of all, though the flow rate through the shunt did decrease as the hydrostatic pressure decreased, it typically did not do so in a linear fashion. In fact, the relationship between pressure and flow rate, as shown in previous flow studies and several of the current flow studies, seemed to be fairly exponential in nature.

Additionally, depending on the starting pressure of the experiments, the relationship between the flow rate and pressure seemed to vary greatly. For example, when the flow rate was measured at extremely high pressures with a steadily decreasing pressure interval, the relationship between pressure and flow rate appeared more linear in nature. The results of the flow experiments at different pressures seem to indicate that the pore size of the e-PTFE is extremely flexible, and can vary depending on the pressure that is exerted upon the polymer. In order to verify these flow rates, more standard flow experiments should be done with the same device, the same starting pressure, and the same denucleation procedure for every trial.

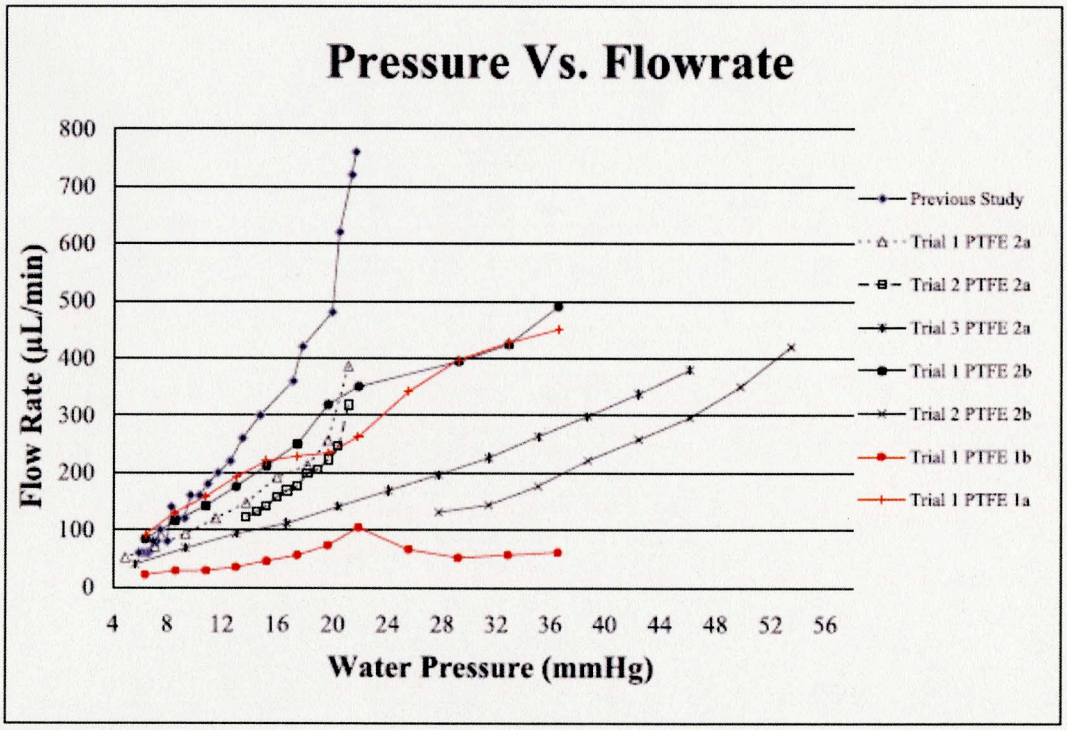

Figure 2.2 Graph showing the flow rate versus pressure for the 1-sided e-PTFE shunts (1a and 1b, red) and the 2-sided e-PTFE shunts (2a and 2b, black)

Flow rate studies were completed on both the 1-sided and 2-sided e-PTFE shunt to evaluate the differences between the different porous surface areas (Figure 2.2). Though in general it seemed as if the flow rate through the 1-sided implant was lower, it was difficult to verify statistically with the variable results that were obtained. Another experiment was completed in which the flow rate through the e-PTFE shunt was measured at different bovine albumin protein concentrations (bovine albumin solid powder was purchased from Sigma-Aldrich). The concentrations tested were chosen based on data showing that the protein concentration can be around 1 g/L in patients with glaucoma, and even up to 10 g/L in cases of inflammation [Prata]. The data from this experiment is valuable because the protein concentration in the inflamed eye of glaucoma patients is much greater than in normal aqueous humor, so determining how the protein concentration affects the flow characteristics of the e-PTFE shunt is useful.

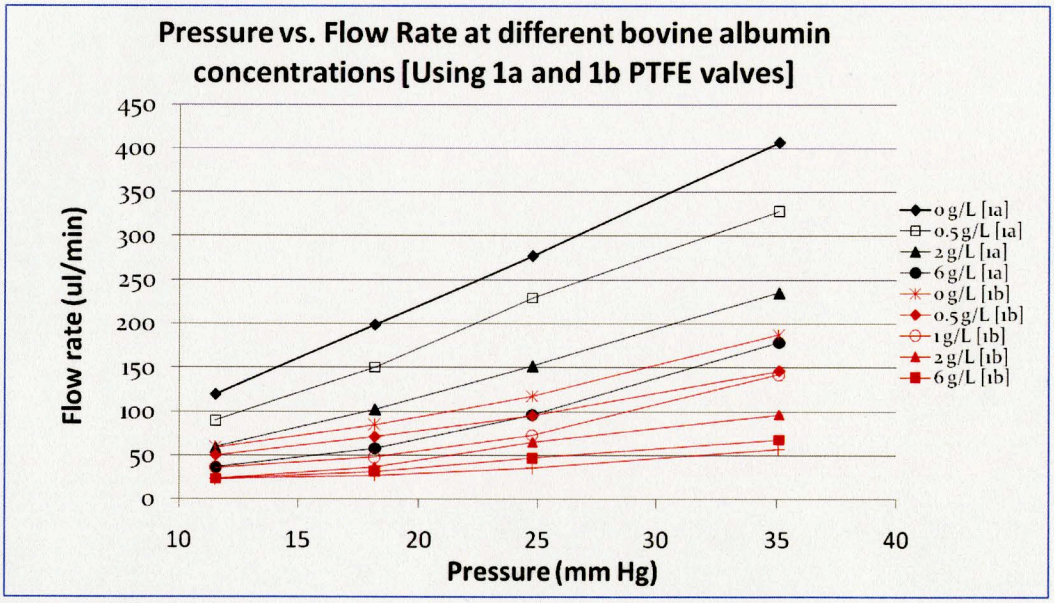

Figure 2.3 Graph showing how the pressure changes with flow rate at different bovine albumin concentrations in both the 1a glaucoma shunt (black) and 1b glaucoma shunt (red)

The results of the protein concentration versus flow rate experiment show a clear decrease in flow rate as the protein concentration was increased, with the effect leveling off at higher protein concentrations (Figure 2.3). Though the results are somewhat unexpected due to the relationship between the pore size in the e-PTFE and the bovine albumin protein size, it seems as if the protein molecules still occlude the pores enough to affect the flow of liquid though them. Despite the lower flow rates with increased protein concentrations, the flow rates still exceed the necessary outflow in the eye. Therefore, in practical use the increased protein concentrations are not expected to detrimentally affect the outflow in patients once the device is implanted, though the different liquid densities should be considered more carefully in future flow rate experiments. In conclusion, it was shown that the flow rate though the valve decreased with increasing protein concentration and that the pore size in addition to the flow rate could be affected by pressure. Future studies with the device should also look at the reservoir pressure of the bleb, since this decreases the outflow in glaucoma drainage devices. The backpressure from the bleb could be taken to account by measuring the flow rate when there is a hydrostatic pressure on the device itself, which could be mimicked by placing the device at a specific depth in a known amount of liquid.

The e-PTFE device is a novel drainage implant with many benefits that could be advantageous for the treatment of glaucoma. Though other implants do show some promise, there are many aspects of the e-PTFE device that make it a potentially more effective option. Some of the other new drainage devices currently on the market will be discussed in the next section.

3. AQUEOUS DRAINAGE DEVICES

3.1. Standard Drainage Devices and Success Rates

There are two major types of glaucoma shunts: valved and non-valved. "The rational of a valve device is to provide a minimal amount of flow resistance, preventing hypotony by creating a 'cut-off switch' to stop flow when a certain IOP is reached" [OphthalmologyWeb]. There is no consensus on which conventional device has the best IOP control with the least amount of complications because studies show conflicting results. However, it has been shown through several experiments that a higher surface area offers lower IOP values, at least in the short term. "The overall success rate among five implants studied, namely, Molteno single and double plate, Baerveldt, Ahmed, and Krupin implants, was between 72% and 79%. All five implants decrease the pressure by 51-62%. There were no statistically significant differences in either the percentage change in intraocular pressure (IOP) or the overall surgical success rate among the five implants." [Shaarawy] More specific information about these conventional aqueous drainage devices can be found in Appendix D.

Comparing different success rates following glaucoma surgery is difficult because the definition of success rate changes between experiments and there are many confounding factors such as the use of medications, the equipment used, the surgeons, prior glaucoma surgery, and the patients themselves. Though controversy still exists on whether or not shunts are preferable to conventional trabeculectomy, many new devices are under development that could be groundbreaking therapies for glaucoma. Also, it could be argued that there is more room for innovation and improvement with glaucoma

drainage devices as opposed to conventional surgery. Glaucoma drainage devices potentially hold more promise for future glaucoma treatment than conventional surgical methods, despite some of their disadvantages. Several new devices and implantation approaches are introduced in this section.

3.2. Solx Device

The Solx device is a gold microwafer shunt implanted ab externo from the scleral side that shunts aqueous humor from the anterior chamber to the suprachoroidal/supraciliary space (see Figure 3.1). The gold micro shunt is thought to increase uveoscleral outflow, which in turn would decrease IOP. The device is very thin and made from 24-karat gold, with 19 tubules (10 are closed and 9 are open) to facilitate aqueous humor outflow [Stamper 2011]. "Gold is known to be biocompatible, with no known long-term toxicity in the eye" [Melamed]. A benefit of this device is that the closed channels in the device can be opened using a titanium-sapphire laser beam to increase aqueous outflow.

In addition, the Solx device avoids the formation of a filtration bleb along with the associated complications. It was approved by the FDA in 2008 and is currently in clinical trials. One pilot study showed that 79% of patients achieved an IOP between 5 mm Hg and 22 mm Hg with the gold micro shunt, and 13% did not need glaucoma medication [Melamed]. On average, patients saw a 44% reduction in IOP, an average IOP of 15.4 mm Hg, and an average of 0.4 medications at their last follow-up [Stamper 2011]. Several complications seen with this device were synechia, hyphema, and retinal

detachment. The results of preliminary studies with this device are very encouraging, and the new suprachoroidal surgical approach may be better than the subconjunctival surgical approach. However, though gold is biocompatible this device would not give the same flexibility in movement as the e-PTFE device.

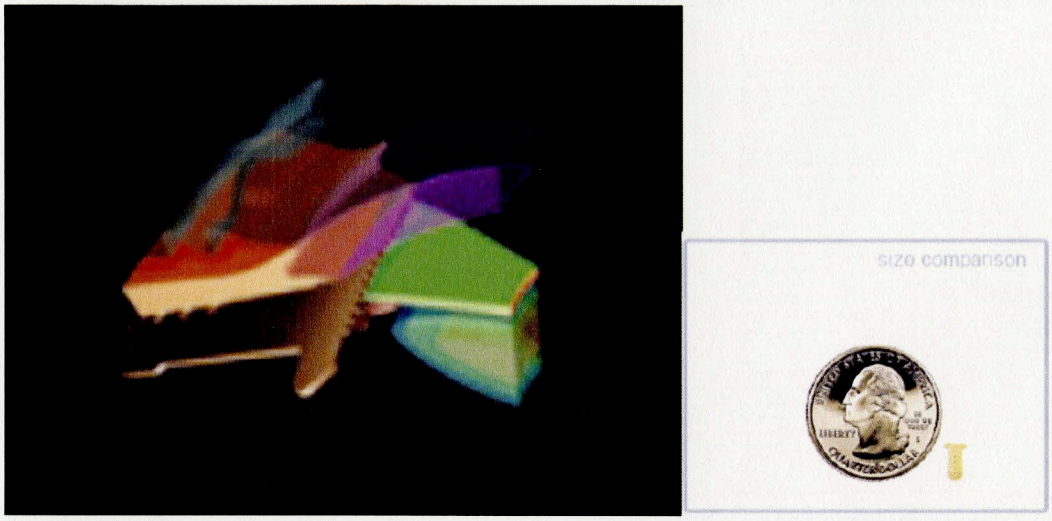

Figure 3.1 Solx device implantation method in the suprachoroidal space (left) and size comparison with a quarter (right) [SOLX Inc.]

3.3. Ex-PRESS Shunt

The Ex-PRESS shunt was approved for use in glaucoma drainage surgery in 2002, and is made out of 316L stainless steel with a conductive oxide layer that helps inhibit an inflammatory response [De Feo]. The device has a beveled, rounded tip, with a projection at the back end to prevent it from exiting the eye, and a disk-like flange at the other end to prevent it from migrating into the anterior chamber [Mermoud]. The shunt is implanted in a procedure similar to a trabeculectomy, and it bypasses the normal aqueous outflow pathway by creating a channel from the anterior chamber to subconjunctival space [Francis 2011]. See Figure 3.2 for the placement and design of the Ex-PRESS

device. Previous studies on the safety of this device have shown that it induces a favorable healing response in human eyes, with the formation of a thin, fibrous capsule around the device and no scar formation or inflammation [Nyska, De Feo].

A study on the effectiveness of this implant showed it had a success rate (defined as IOP less than 21 mm Hg) of 76.9% at the patient's last visit (usually a 3 year follow-up) [Traverso]. Another study showed it had a success rate (defined as IOP between 5 and 21 mm Hg with no additional surgery necessary) of 84.3% at 15 months with implantation of the Ex-PRESS device [Maris]. As with other shunt procedures, there is the risk of trauma to the cornea or iris and it still requires the use of antifibrotics or other glaucoma medications. In addition, though the stainless steel has an oxide layer to reduce inflammation, there would still be issues with inflammation, fibrotic growth, and surrounding tissue necrosis. These same issues are not encountered with the use of the e-PTFE shunt.

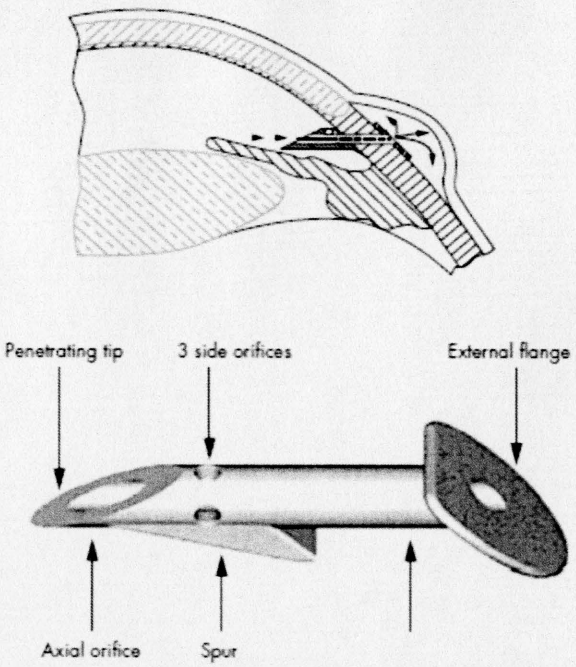

Figure 3.2 Placement of the Ex-PRESS shunt in between the anterior chamber and subconjunctival bleb (top), and design of the Ex-PRESS shunt (bottom) [Traverso]

3.4. IStent Implant

An L-shaped nonferromagnetic titanium tube called the iStent is another novel device that can be used in glaucoma filtration surgery. "The trabecular micro-bypass stent is designed to create a permanent opening from the anterior chamber into Schlemm's Canal" [Francis]. The shunt is designed to fit and stay in Schlemm's canal, and one end is pointed for insertion into the canal while the other end has a half cylinder over it to prevent blockage of the tip (see Figure 3.3 for the iStent shunt design).

A very small pilot study showed that the device "warranted further investigation," though the data was limited [Francis]. Another study conducted showed that "compared with cataract surgery alone, implantation of the iStent concomitant with cataract

extraction significantly increased trabecular outflow facility, reduced IOP, and reduced the number of medications at 1 year" [Fernández-Barrientos]. It is difficult to elucidate the exact effect of the iStent alone, however, because it was usually combined with cataract removal, a surgery known to lower IOP.

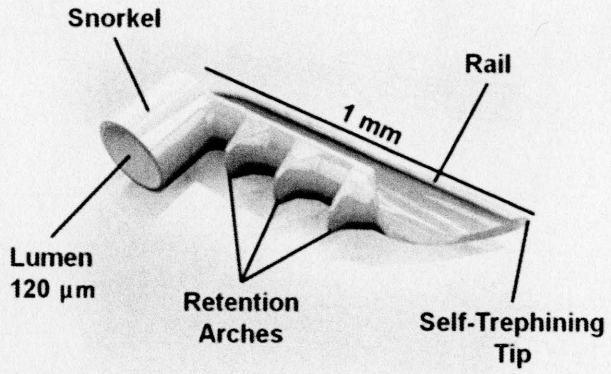

Figure 3.3 Design of the iStent Implant [Harris]

3.5. CyPass Shunt

The CyPass is another novel shunt that can be used for the treatment of glaucoma (see Figure 3.4 for a size comparison between the CyPass device and a dime). It is a tiny "tube-like device made of a highly biocompatible material that is inserted into the suprachoroidal space just above the ciliary face across the anterior chamber" [Stamper 2011]. This technology has been available in Europe since 2009, but is "only available in the U.S. through the COMPASS clinical study" [Transcend Medical]. Though the preliminary studies are currently not published, some results are quoted that show patients having an average IOP of 13 mm Hg, a 35% reduction in IOP, and an average of 0.9 medications at the last follow-up [Stamper 2011].

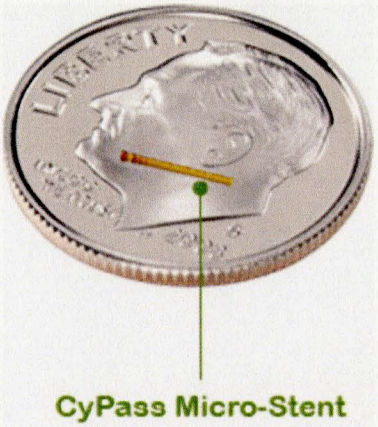

Figure 3.4 Size comparison between the CyPass implant and a dime [Transcend Medical]

In order to improve surgical outcomes certain factors such as placement and surgical method need to be considered. Recent studies have shown the importance of the lymphatic networks near the eye following drainage surgery, so developing a surgical technique that preserves the lymphatic networks near the eye could be very beneficial. An *in vivo* rabbit study to begin assessing the role of the ocular lymphatic channels in aqueous humor outflow was performed. The rabbit study looking at a new surgical approach is expounded upon in the next section.

4. *IN VIVO* RABBIT STUDY

4.1. When is Glaucoma Surgery Necessary?

Despite the challenges with conventional filtration surgery, there are numerous reasons why an improved surgical technique as a first line of therapy would be beneficial. As stated previously, most patients throughout the world who have glaucoma do not have access to drugs or cannot afford them. Therefore a safe, permanent surgical procedure that could be performed with minimal equipment is needed. In addition, if surgery could be used as the sole treatment for glaucoma there would be no issues with patient adherence and the onetime cost would make therapy much more accessible. However, if glaucoma surgeries were to be performed in third world countries, there are some practical aspects to consider. For example, a system would need to first be in place to properly train surgeons in order to prevent infection and other complications. These challenges are not insurmountable. The surgical method explored in this study could be easier to perform and more effective, making it accessible to more glaucoma patients.

4.2. Introduction of Experiment

The main goal of this experiment was to identify a surgical technique that preserves the lymphatic channels near the eye. Though in normal eyes there does not seem to be a connection between the lymphatics of the conjunctiva and aqueous humor outflow, this appears to change drastically after glaucoma surgery [Singh]. The conjunctival lymphatics play a major role in aqueous humor outflow once surgery has been performed, and therefore it is extremely essential that the surgeon protects these

lymphatic networks during surgery [Yu]. Up until very recently there has been very limited data in the literature concerning the conjunctival lymphatics along with its role in aqueous humor outflow [Singh].

The lymphatic networks of the eye should definitely be studied in more detail, especially since their importance has recently been highlighted. In previous studies, the lymphatic channels near the eye have been shown to run perpendicular to the limbus [Yu, Singh]. Thus, in this experiment we will perform glaucoma drainage surgery by making an incision perpendicular to the limbus (called vertical in this experiment) in one group and an incision parallel to the limbus (called horizontal in this experiment) in another group. In this way we hope to compare the two surgical methods and show that the outcome of the surgery is drastically improved when the surgical incisions are completed in parallel to the lymphatic networks of the eye as opposed to directly across. Our hypothesis is that the surgery employing the vertical incision will more effectively preserve the lymphatic networks when compared with the horizontal surgery.

4.3. Justification of Experiment

A new surgical method using a microfistula shunt was employed by Yu et. al in both rabbits and monkeys. In the animals where lymphatic drainage pathways were clearly visible, the aqueous humor drainage pathway remained effective for a longer period of time. Therefore, the authors clearly showed the importance of the conjunctival lymphatic network in the overall success of glaucoma drainage surgery. Due to the nature of this experiment, and after an extensive literature search, it was concluded that

performing this experiment using a computer model or *in vitro* techniques would not yield the desired information. We are exploring a surgical method and its effects on an entire physiological system. Therefore, *in vitro* methods would not accurately show the interaction between the different tissue types and their response as a unit to the trauma of surgery. Because of the complexity of the system, and the unknown factors, creating a computer model to accurately predict the behavior of the eye is currently impossible.

In planning this experiment we had considered using animals smaller than rabbits, such as rats or mice. However, since we would like to apply the information obtained from this study to humans we decided to use an animal model that more closely approximates a human eye. Therefore rabbits were chosen since the size and structure of a rabbit eye lends itself more easily to glaucoma drainage surgery and comparison with human eyes. There is also a rabbit model of glaucoma that could be very useful in this study, while there is no rat or mouse model of glaucoma. Potential candidates for glaucoma models that could be used are the genetically predisposed buphthalmic rabbits or rabbits that have glaucoma induced by alpha chymotrypsin A [Gelatt]. Additionally, in practice performing this type of glaucoma filtration surgery on an animal such as a mouse would be extremely difficult and impractical. Fluorescent imaging of vascular and lymphatic networks would also pose more of an issue in the rat and mouse models than in the rabbit model.

Though this work partially duplicates a previous study, the surgical techniques that we will test are different. In the previous study the researchers performed a very precise glaucoma drainage surgery employing a microfistula shunt. For general use, this

method would require the attention of an extremely skilled surgeon along with very expensive equipment. The importance of the surgical techniques that we tested is that they are accessible to more people and will be able to be performed by any opthalmic surgeon. The method for glaucoma drainage surgery that we hoped to identify will be extremely practical in its application.

4.4. Materials and Methods

Materials

Five rabbits were used for this pilot study. The rabbits were purchased from Harlan laboratories, and were acclimated to the University Animal Care Center for one week before experimentation. Trypan blue dye bought from Sigma-Aldrich was used in the imaging portion of the experiment. A small section (~2 mm) of a silicone tube was used as a glaucoma shunt. Various microsurgical tools, opthalmic knifes, speculum, balanced salt solution, 23 gauge needles, and absorbable adle-vicryo sutures were used during surgery and imaging. Everything used during surgery was either heat sterilized in an autoclave (metal objects) or chemically sterilized using ethylene oxide gas (silicone or plastic). During surgery a glass bead sterilizer was used to keep the surgical tools sterile.

Surgery

NIH guidelines for the care and use of laboratory animals will be observed throughout the study (NIH Publication #85-23 Rev. 1985). In addition, this procedure was performed under IACUC protocol #10-241 at the University of Arizona Animal Care Center. Rabbits were anesthetized using an intramuscular injection with 0.5 cc/kg of a

rabbit mix (solution mixed is 2 mL of 10 mg/mL acepromazine, 5 mL of 100 mg/mL ketamine, and 8 mL of 20 mg/mL xylazine). Betadiene was sprayed around the eye, which was held open with a wire eyelid speculum. Proparacaine drops (1-2 drops) were administered to the surface of the eye, and then epinephrine was put onto the eye with a cotton swab. Either a vertical or horizontal incision was made in the conjunctiva using a super sharp ophthalmic knife, and then the flap was opened farther by using forceps to hold it up and then cutting with scissors. After, a stab incision was made from the vertical or horizontal incision near the limbus to the anterior chamber.

The silicone tube was then inserted into the new pathway made to the anterior chamber. Once the silicone tube was in place, and drainage of aqueous humor was visible, the end of the silicone tube was cut down to size. Balanced salt solution was injected in the anterior chamber to prevent collapse of the anterior chamber. The conjunctival flap was then closed with a vicryl suture, the speculum was removed, and a drop of zymar was put on the eye. Then, the eyes were treated with a topical antibiotic and analgesic for 4 days post-operatively. The antibiotic used was neomycin ointment while the analgesic used was proparacaine. IOP measurements were made with a tonopen (Reichert) periodically throughout the study.

Histology

After five weeks, the rabbits were euthanized and the eyes were removed and fixed for histological examination. Paraffin sections 5 μm thick were stained with hematoxylin and eosin for evaluation of overall tissue organization. Other serial sections were stained using immunohistochemical staining. The CD34 antibody was used to stain

vascular endothelium, in order to be able to count the number of nearby vessels. Then, CD31 was used to stain both the vascular and lymphatic endothelium to count the total number of vessels. The D2-40 antibody was also used to specifically stain the lymphatic endothelium. All of the primary antibodies were mouse antibodies, and all the secondary antibodies were anti-mouse rabbit antibodies. The immunohistochemical staining was performed in Dr. Raymond Nagle's pathology laboratory at the University of Arizona Cancer Center.

Imaging of lymphatics and vasculature

During the imaging studies, the rabbits were anesthetized using an intramuscular injection with 0.5 cc/kg of a rabbit mix (solution mixed is 2 mL of 10 mg/mL acepromazine, 5 mL of 100 mg/mL ketamine, and 8 mL of 20 mg/mL xylazine). Then, the trypan blue was administered using a 23 gauge needle in the anterior chamber of all of the eyes, and into the subconjunctival tissue of several eyes (rabbits 11-03 and 11-05). Using a technique similar to that previously described by Seetner and Morin, trypan blue was injected into the subconjunctival tissue to create interstitial tissue fluid, which then can drain through the lymphatics. A 23-gauge needle was used to inject sterile trypan blue to form a small subconjunctival blister just below the conjunctival epithelium [Yu]. The pool of trypan blue was be observed in order to evaluate if it remained stagnant, meaning lack of lymphatic drainage, or if it spread away from the blister, indicating the existence of lymphatic drainage and showing the distinct branch-like tributaries. The rabbits were then observed for several hours after injection into the anterior chamber and

subconjunctival tissue. The day after the imaging studies were completed, the rabbits were euthanized using an intracardiac injection of 150 mg/kg of sodium pentobarbitol.

Experimental Setup

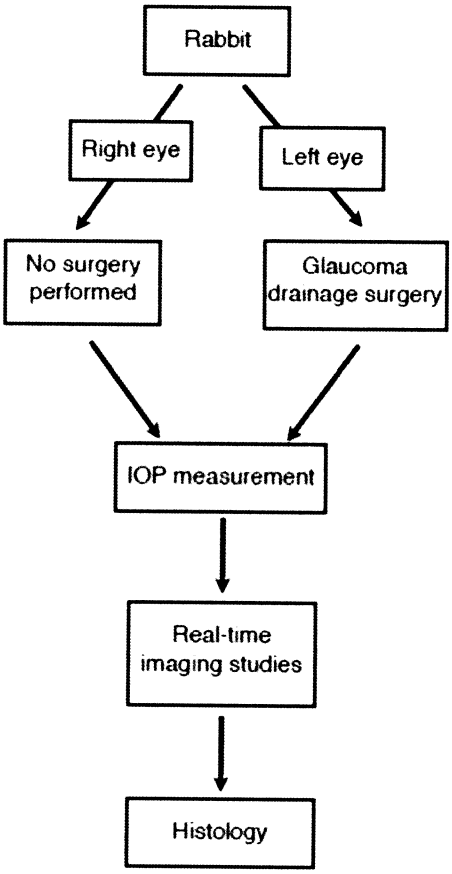

Figure 4.1 Experimental Setup

Five rabbits were used during this study, and surgery was performed in the left eye of each rabbit while the right eye of each rabbit was left as a control (Figure 4.1). Two different types of surgery were completed to test their effects: vertical incision near the limbus and horizontal incision near the limbus. Rabbit 11-01 had a vertical incision, rabbit 11-02 had a vertical incision, rabbit 11-03 had a vertical incision, rabbit 11-04 had a horizontal incision, and rabbit 11-05 had a horizontal incision.

4.5. Imaging Study Results and IOP Results

Several pictures were taken during the surgery to show the method of implantation used in this study for the glaucoma shunt.

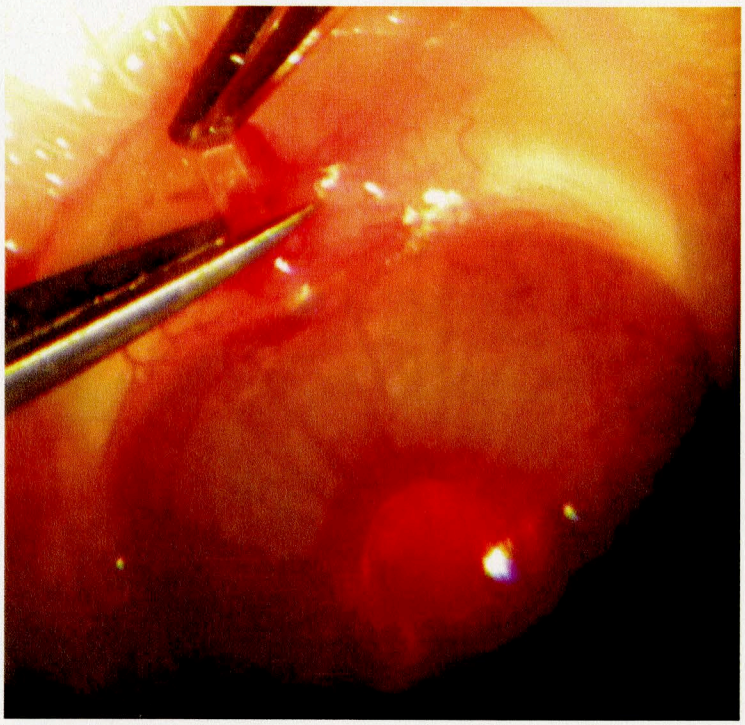

Figure 4.2 Image showing the placement of the silicone tube in a rabbit eye during surgery, from the limbus into the anterior chamber, using forceps

After surgery, the IOP of the rabbits was measured at 9 days (Table 4.1). The IOP measurements show that the surgery was effective in lowering IOP. With a visual inspection, at 9 days the glaucoma shunts and bleb formation could be seen in most of the rabbits. The IOP was also measured prior to performing the imaging studies. These results showed that the pressure of the rabbit's eyes greatly decreased after being put

under anesthesia, ranging from 5 mm Hg to 18 mm Hg. In addition, at 5 weeks the shunts were not visible when the eyes were observed.

Rabbit #	Right eye IOP (no surgery)	Left eye IOP (surgery)	Visual Appearance
11-01	21	18	Small bleb, hard to see tube in anterior chamber
11-02	24	19	Tiny bleb, can see tube in anterior chamber
11-03	29	26	Tube is in place and visible
11-04	25	21	No bleb, but the tube is visible
11-05	23	19	Shallow bleb, can see tube in anterior chamber

Table 4.1 IOP data from the rabbits (in mm Hg), 9 days following surgery

Imaging studies were performed on the rabbits at 5 weeks following the surgery, prior to euthanization (Figure 4.3). After the trypan blue was injected into the anterior chamber of the rabbits, they were observed for several hours. The perfusion out of the anterior chamber was not visible in specific blood vessels, but the sclera appeared to be a diffuse blue color in the eyes on which surgery was performed (Figure 4.4).

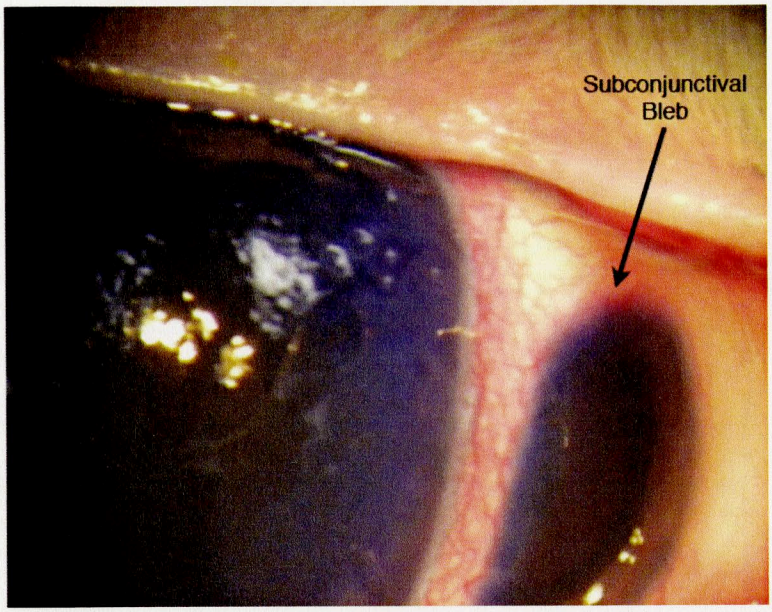

Figure 4.3 Anterior chamber and subconjunctival bleb filled with trypan blue at 5 weeks following surgery, during the imaging studies on the rabbits

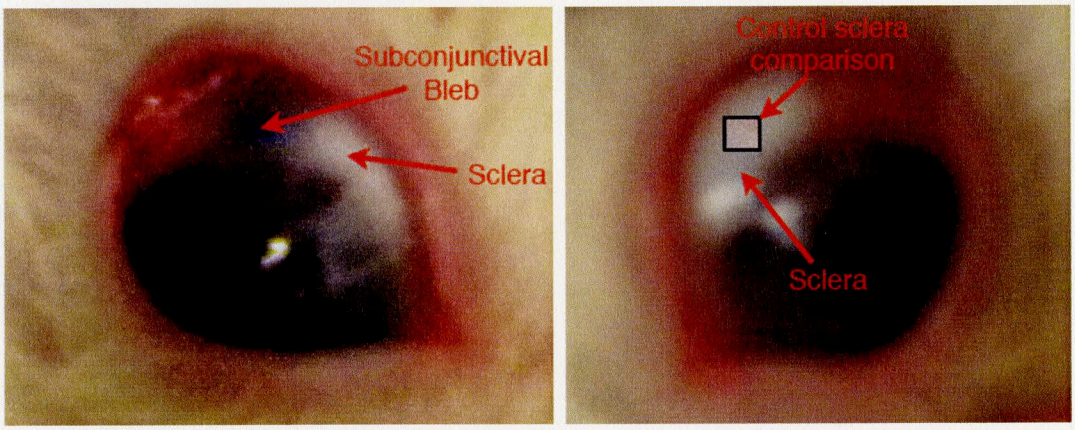

Figure 4.4 Comparison of control right eye with a subconjunctival bleb (left) to a surgically treated left eye with a blue-tinted sclera (right)

Following euthanasia, another imaging study was performed to ensure visualization of trypan blue perfusion through the vessels since it was difficult to see in the initial imaging study. The setup for the continuous perfusion imaging study was: a syringe connected to a tube with a 23 gauge needle at the end that was inserted into either

the anterior chamber or subconjunctival space of the rabbit eye. The pressure was adjusted by changing the height, and was set between 15 and 20 mm Hg to mimic physiological pressure. With this setup, perfusion of the trypan blue into surrounding vessels was visible. It appeared that some vessels that filled with trypan blue were conjunctival lymphatics because they were distinct from some of the visible surrounding blood vessels, and they moved when the conjunctiva was manipulated. Pictures showing perfusion in the rabbit eyes during the second imaging study are shown from Figure 4.5 to Figure 4.9.

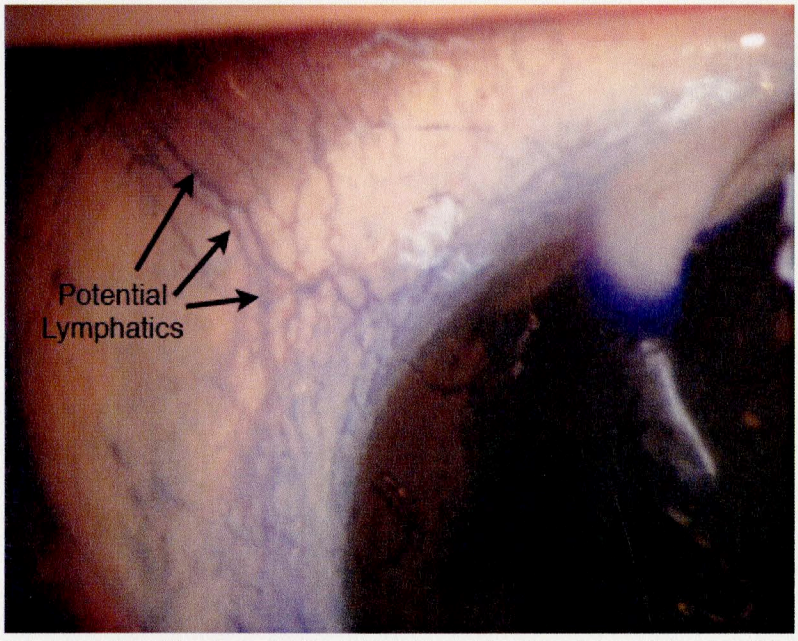

Figure 4.5 Image showing perfusion of trypan blue in the right eye of rabbit 11-01 (control)

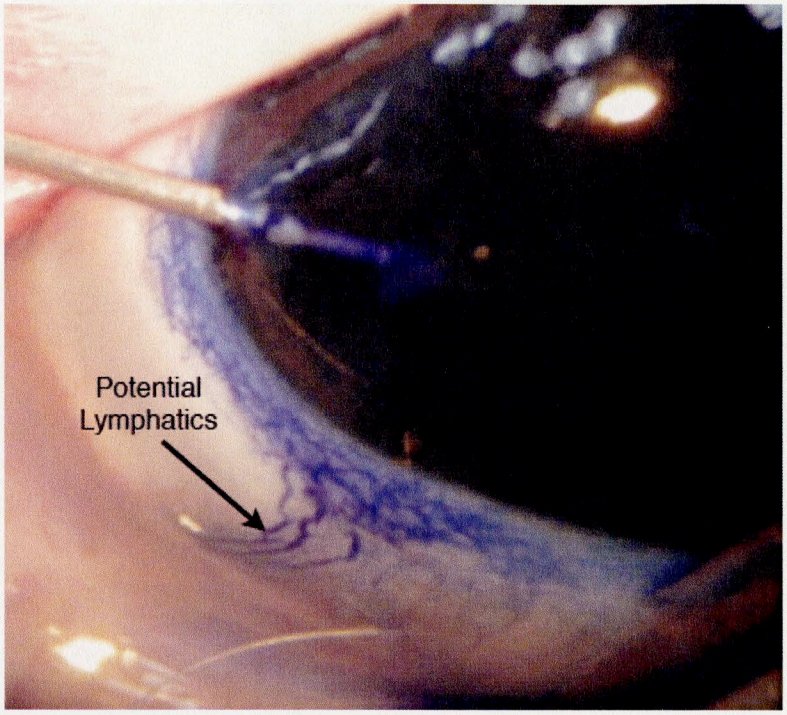

Figure 4.6 Image showing perfusion of trypan blue in the left eye of rabbit 11-01 (vertical incision)

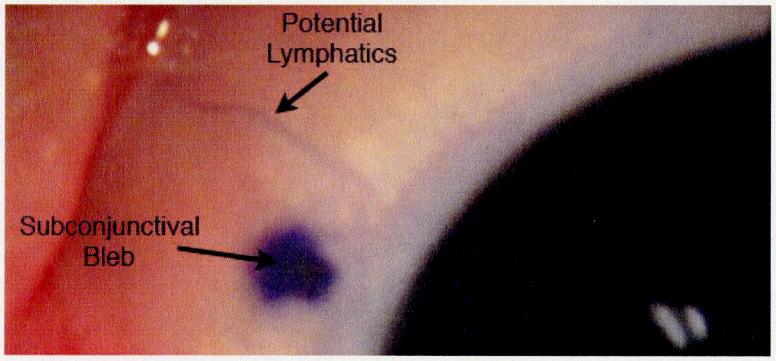

Figure 4.7 Perfusion of trypan blue in a subconjunctival bleb in the right eye of rabbit 11-03 (control)

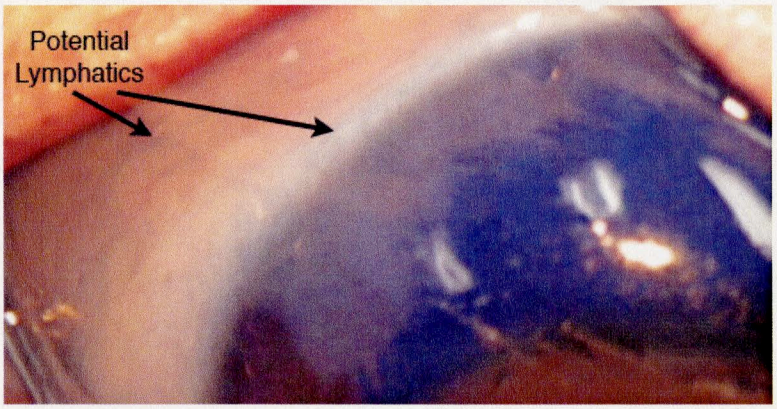

Figure 4.8 Image showing perfusion of trypan blue in the right eye of rabbit 11-04 (control)

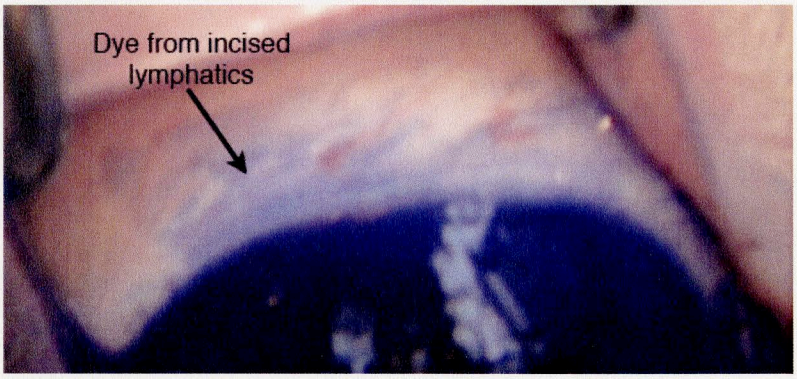

Figure 4.9 Image showing perfusion of trypan blue in the left eye of rabbit 11-04 (horizontal incision)

4.6. Histology Results

The hemotoxylin and eosin stained histology slides showed the overall tissue organization in the rabbit eyes. Tissue sections were taken in cross section from the limbus, near the border of the sclera and cornea. Figures 4.10 to 4.13 show some H&E histology slides taken from the left and right eye of rabbit 11-04. Certain anatomical structures of the eye can be seen in the H&E slides, including the ciliary process, the conjunctiva, the sclera, and the lens. The histology slides did not show any conclusive

difference between the different types of surgery or between surgically treated eyes and control eyes. However, there is evidence of lymphocyte infiltration into the tissue, as can be seen by the potential lymphatic cells (tiny purple circles) between the cells in the tissue of the sclera and the conjunctiva.

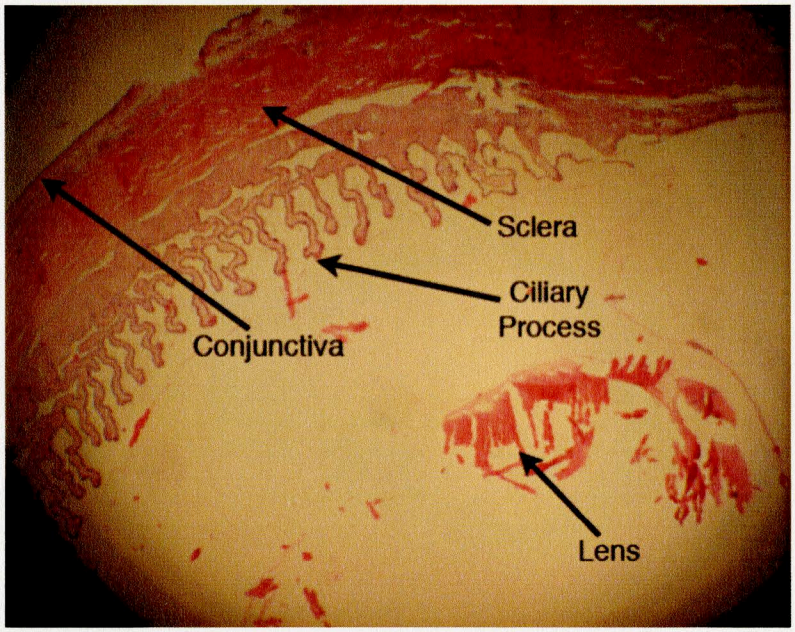

Figure 4.10 Histology sections (stained with Hemotoxylin and Eosin) from the right eye of Rabbit 11-04, at 2x magnification

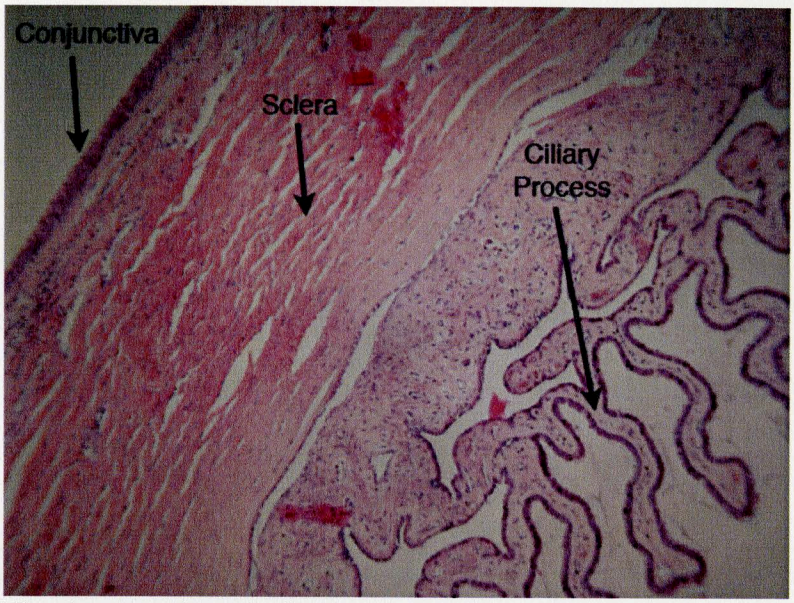

Figure 4.11 Histology sections (stained with Hemotoxylin and Eosin) from the right eye of Rabbit 11-04, at 10x magnification

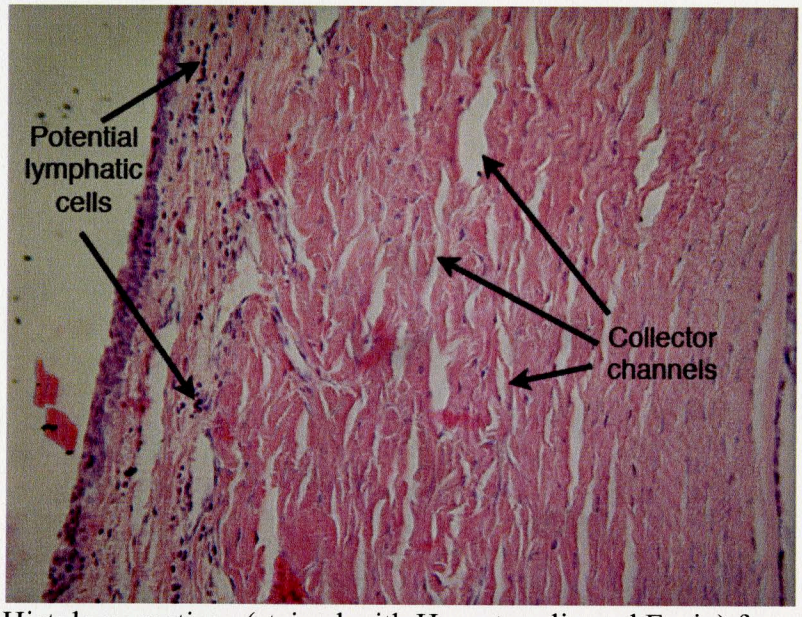

Figure 4.12 Histology sections (stained with Hemotoxylin and Eosin) from the right eye of Rabbit 11-04, at 20x magnification

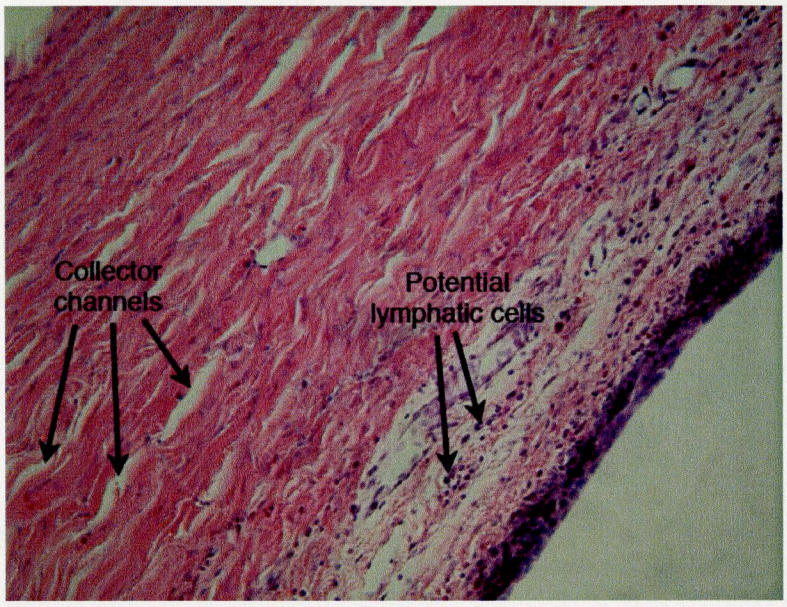

Figure 4.13 Histology sections (stained with Hemotoxylin and Eosin) from the left eye of Rabbit 11-04, at 20x magnification

Staining to see the blood vessels was also performed using the CD34 antibody, but the antibody did not function properly because it did not stain the positive control. Therefore, the results of that staining were invalid and the staining needs to be repeated. In addition, immunohistochemistry staining was completed with the lymphatic vessel specific D2-40 primary mouse antibody along with the lymphatic and blood vessel specific CD31 primary mouse antibody. A secondary anti-mouse rabbit antibody was used to detect the presence of the primary antibody, via a connected peroxidase enzyme that turns a reddish-brown color in the presence of a substrate.

Due to the fact that a secondary rabbit antibody was used, there was a great deal of background staining. Compared with the positive controls, which show very specific binding to the vessel endothelium (see Figure 4.14 and Figure 4.16), the stained samples show a lot of excess staining (see Figure 4.15 and Figure 4.17), and the negative controls

also show staining where they should not (see Figure 4.18). Therefore, per my conversation with the pathologist Dr. Raymond Nagle at the Arizona Cancer Center who viewed the slides for scoring, no conclusive statement could be made regarding the presence of lymphatic or blood vessels. Staining with a more specific antibody should be completed in the future to verify the presence of lymphatic vessels. A secondary antibody that is not from a rabbit should be used in the future when staining rabbit tissue.

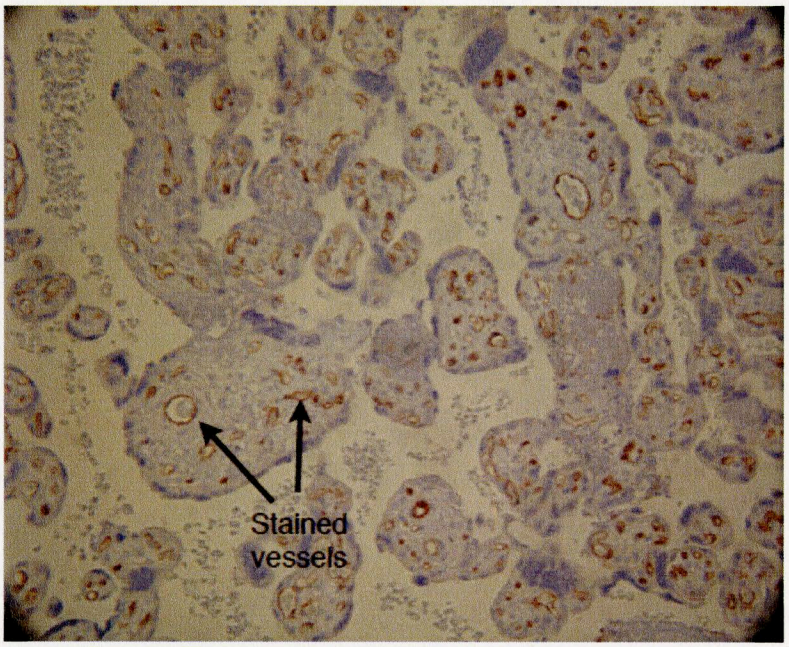

Figure 4.14 Histology sections stained with CD31 blood and lymphatic vessel-specific antibody from a human placental tissue positive control, 20x magnification

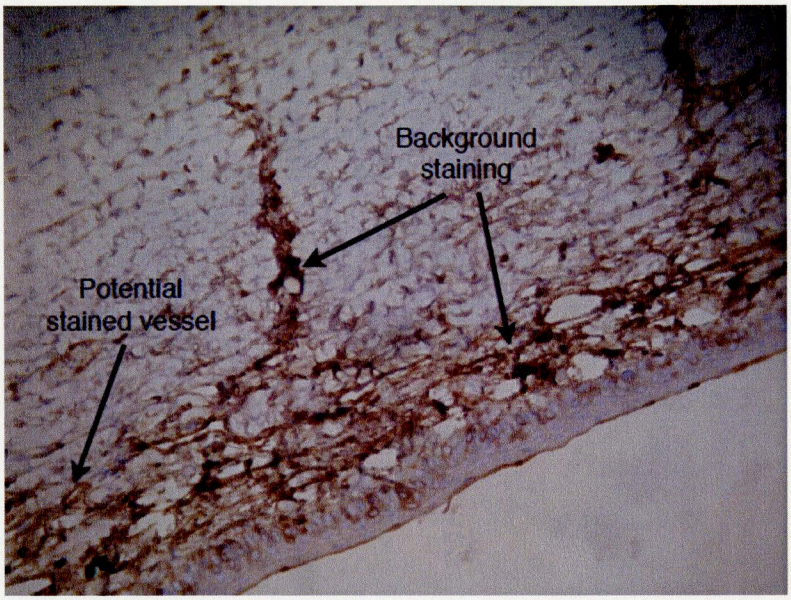

Figure 4.15 Histology sections stained with CD31 blood and lymphatic vessel-specific antibody from the right eye of Rabbit 11-04, 20x magnification

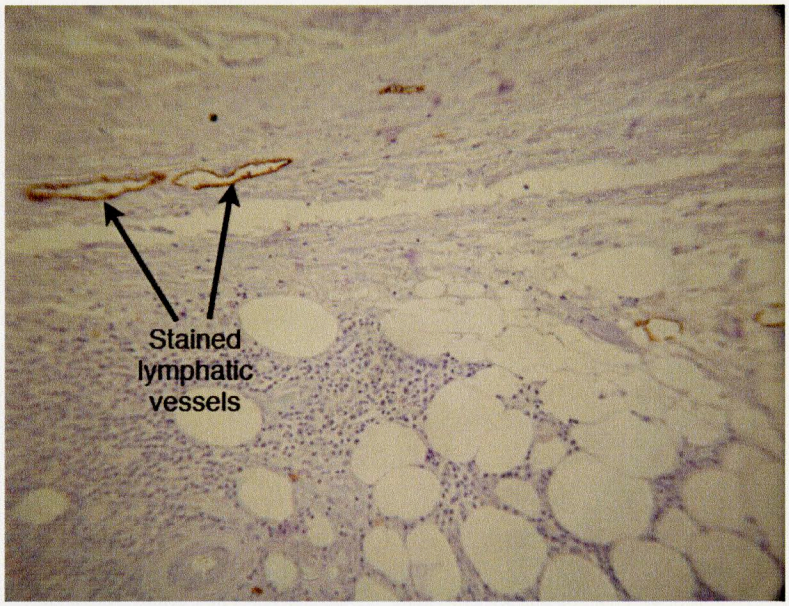

Figure 4.16 Histology sections stained with D2-40 lymphatic vessel-specific antibody from a human placental tissue positive control, 20x magnification

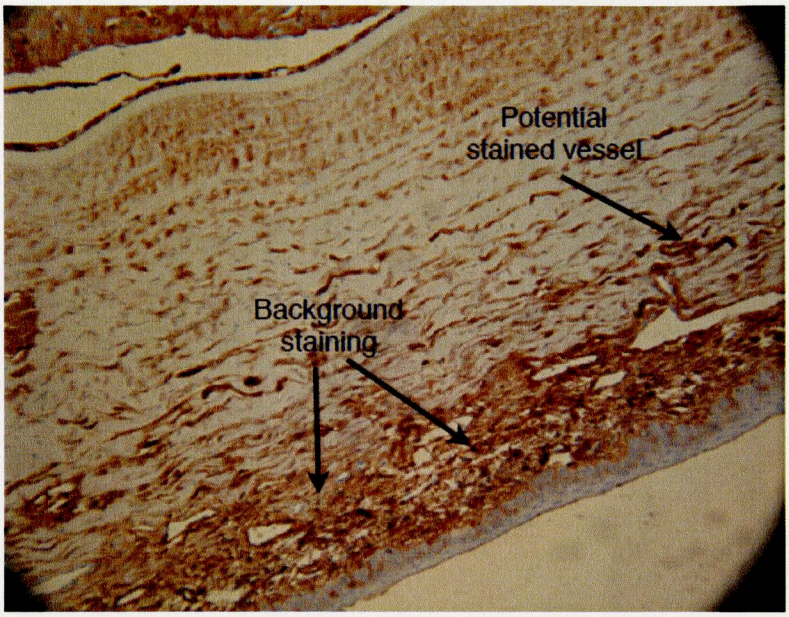

Figure 4.17 Histology sections stained with D2-40 lymphatic vessel-specific antibody from the right eye of Rabbit 11-04, 20x magnification

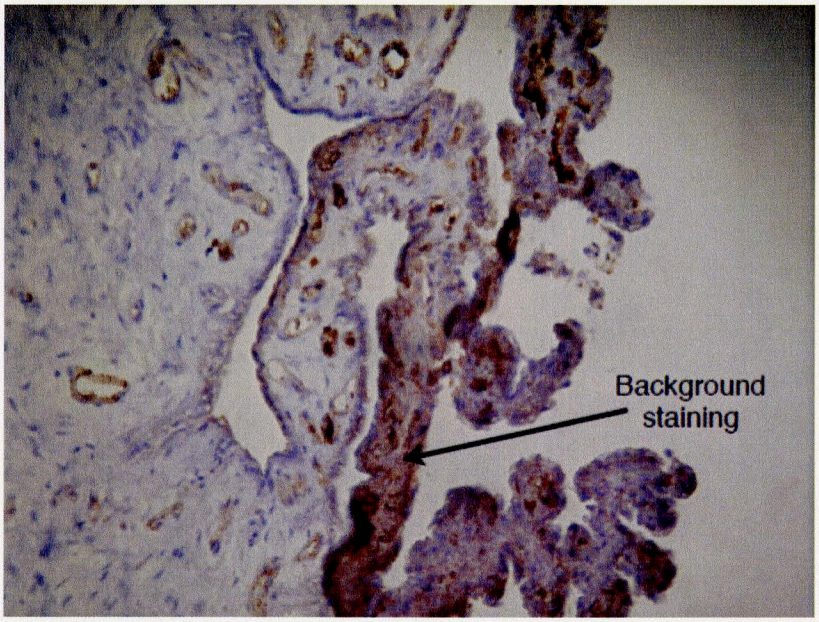

Figure 4.18 Negative control stained with the secondary anti-mouse rabbit antibody from the right eye of Rabbit 11-04, 20x magnification

4.7. Discussion

The glaucoma drainage surgery and subsequent imaging showed that lymphatics are potentially involved in the outflow of aqueous humor after surgery. The lower IOP in the eyes with the glaucoma drainage devices proves that the surgery successfully reduced the IOP. During the first imaging study, while the rabbits were still alive, perfusion of trypan blue into the surrounding vessels was not visible. However, the sclera in the surgically manipulated eyes appeared to be a diffuse blue color for a couple hours after the initial injection of the trypan blue into the anterior chamber. The blue tint to the scleral tissue seems to suggest that there is outflow from the anterior chamber into the subconjunctival space (though the dye did not stay in one contained bleb), bypassing the traditional aqueous humor outflow pathways. There are several reasons why specific vessels may not have been highlighted by the trypan blue.

One reason for the lack of proper perfusion could be that the natural aqueous production in the rabbits' eyes could have diluted the dye a great deal, making it not concentrated enough to show up clearly. In addition, when the rabbits were under anesthesia their IOP was extremely low, which could have prevented aqueous humor outflow until the pressure returned to normal. The shunts had most likely scarred over because they were not visible. If the shunts were not functioning properly, then the aqueous humor perfusion would be difficult to see. All in all, the initial *in vivo* imaging study with trypan blue shows diffusion into the subconjunctival space in eyes that underwent placement of a glaucoma implant, which makes sense in light of the fact that

the new drainage pathway created by the surgery shunts aqueous humor from the anterior chamber to a bleb underneath the conjunctiva.

After the rabbits were euthanized, trypan blue was perfused at a constant pressure into the anterior chamber of all five rabbits, and into the subconjunctival space of both eyes of rabbit 11-03. The hypothesis of this experiment was that the lymphatic networks are better preserved when surgery is performed along the lymphatic networks instead of directly across the lymphatic networks. Since the lymphatic networks have been shown to run perpendicular to the limbus, the rabbits that underwent surgery with a vertical incision should show better lymphatic perfusion during these flow rate studies. Rabbits 11-01, 11-02, and 11-03 all had a vertical surgery (perpendicular to the limbus) while rabbits 11-04 and 11-05 both had horizontal surgery (parallel to the limbus).

Though the results from the pictures are not entirely conclusive, there does seem to be a pattern showing that the lymphatic networks are cleaved more during the horizontal incision surgery. In all of the right eyes, several different randomly oriented vessels are visible with trypan blue, some of which seem to collect near to the limbus (Figure 4.5 and 4.8). The vessels filled with trypan blue seen in the photos of the right eyes may be lymphatics, as there are a couple instances where separate blood vessels are also visible (Figure 4.8) or the structure is indicative of lymphatics (Figure 4.5). Therefore, the images of the control eyes potentially show how lymphatic perfusion in normal eyes would appear. In all the left eyes of the rabbits that underwent vertical incision surgery, there was clear evidence of normally arranged, intact lymphatics. Additionally, in rabbit 11-01 the potential lymphatic networks appear very dark and

concentrated around one particular area, hinting that those particular lymphatic channels may have been near the shunt and utilized for increased aqueous humor outflow (Figure 4.6).

In contrast, the dye in each left eye of the rabbits that went through horizontal incision surgery did not seem to perfuse into distinct collector vessels in the same manner as the vertical incision group. The trypan blue is visible in the left eyes of rabbits 11-04 and 11-05 as a darker blue coloring over sections of the sclera, hinting that the lymphatics were not kept intact during surgery (Figure 4.9). Without whole lymphatic vessels, the dye could diffuse underneath the conjunctiva instead of being taken up by unaffected collector vessels. The results that show potential lymphatic activity need to be further verified with immunohistochemical staining, which needs to be repeated due to the inactive antibody or excessive background staining. The H & E staining did not show any visible difference between the tissue of the surgically treated eyes and the control eyes or any difference between the types of surgery. However, the staining did show potential lymphatic infiltration in almost all of the samples, indicating that the lymphatics do play a role in the functioning of the eye. More work needs to be done to verify that the lymphatic cells visible in the slides are related to aqueous humor outflow.

A dye that could stay perfused in the tissue during the histological studies would help in distinguishing lymphatic vessels from blood vessels during the imaging studies. This type of dye would allow for direct comparison of the unknown vessels filled with dye *in vivo* and those highlighted as lymphatics with immunohistochemical staining. Also, a quantitative measure of the number of vessels perfused, in both the imaging study

and the histology slides, or the actual area of diffuse blue perfusion would help with comparisons. Immunohistochemistry staining to see intact lymphatic endothelium versus broken lymphatic endothelium would help confirm destruction of the lymphatic vessels in the horizontal incision samples. Future studies should use a larger number of animals, more than 2 rabbits per type of surgery, in order to verify results.

In summary, the imaging studies hinted at the involvement of lymphatic networks in the aqueous outflow pathway created with the shunt, and suggested that the horizontal incision surgery is more destructive to the lymphatic vessels of the conjunctiva. Other new surgical techniques have been developed recently, and though they some show promise none seem to account for the role of the lymphatics. Various innovative surgical approaches are talked about next.

5. SURGICAL GLAUCOMA TREATMENT

5.1. Introduction

In some patients, pharmaceutical treatment of glaucoma does not reduce the IOP enough to prevent vision loss. Additionally, glaucoma medications are not tolerated by some patients because of their side effects, or are an unrealistic option depending on the patient's social and economic situation [Dietlein]. When drug therapy fails, surgery is another option for patients suffering from glaucoma. Currently, in the United States, surgery is usually only considered when necessary because of the many complications that can arise. The risk of complete blindness in the first year after surgery is very low, but 20% to 30% of patients who undergo surgery experience loss of central visual acuity due to cataracts, macular edema, or retinal wrinkling. These complications should be addressed if surgery is used as the primary treatment. There are many different types of surgery, but they fall into two main categories: conventional and laser surgery. The surgical option recommended for a patient varies depending on the type of glaucoma they have. In this section, the frequently performed trabeculectomy along with some newer surgical techniques are introduced. Though some of these current methods have decent success rates, they usually destroy the lymphatic channels that could be essential in aqueous outflow following surgery.

5.2. Trabeculectomy

The most common type of surgery performed for the treatment of glaucoma is a trabeculectomy. During a trabeculectomy a small section of the trabecular meshwork is

removed in order to create an opening to drain aqueous humor [Watson, MedicineNet]. "Direct modification of the outflow pathway surgically is justified by the hypothesis that the majority of outflow obstruction in primary open-angle glaucoma (POAG) lies in the juxtacanalicular trabecular meshwork (TM) or inner wall of Schlemm's canal (SC)" [Francis 2006]. The new surgically created outflow pathway opens into a pocket in the subconjunctival space called a filtering bleb. Collector vessels are thought to transport aqueous humor out of the bleb and into systemic circulation. The bleb is necessary to prevent the dangerously low IOP that can result from direct contact between the inside of the eye and atmosphere. Blebs are extremely important in a trabeculectomy, along with other types of glaucoma drainage surgery, and the state they are in can greatly influence the success or failure of the drainage surgery. In general, however, a bleb-independent surgery would be preferred in order to avoid issues of late bleb failure and bleb infection [Peckar].

"Trabeculectomy is still the most effective IOP-lowering procedure performed today but continues to have the highest serious complication rates," leading to the continual search for alternative surgical procedures [Mosaed]. One particular study showed that a trabeculectomy has a success rate (IOP of less than 21 mm Hg, IOP 20% below baseline in two consecutive visits, and no additional surgery necessary) of 86% one year following surgery [Mosaed]. When compared to other surgical methods, a trabeculectomy is more effective in lowering IOP. After surgery, patients have an average IOP of 12 mm Hg, which is a lower pressure than is typically obtained by using other procedures.

Despite effectively lowering IOP, many complications frequently occur following a trabeculectomy. These include "hypotony, bleb leaks, late blebitis, accelerated cataract progression, choroidal effusions and hemorrhage, and prolonged or permanent visual impairment from hypotony maculopathy" [Mosaed]. The postoperative complications from the surgery, including overfiltration and hypotony, are mainly due to the sudden exposure and resulting decompression of the anterior chamber [Lachkar].

Antifibrotic agents such as mitomycin C or 5-Fluorouracil are commonly used to prevent fibrosis and scarring, which can obstruct the newly created outflow pathway following surgery [Johnson 2001, Dietlein]. Without the use of antifibrotic agents the trabeculectomy procedure has relatively high short-term failure rates [Mosaed]. However, the antifibrotic agents also increase the number of complications that a patient experiences and can be the cause of things such as corneal erosion, corneal ulceration, conjunctival wound leaks, or thin avascular blebs. During the surgery, several complications can also occur, including tearing of the conjunctival flap, hemorrhage, lens injury, vitreous loss, stripping of the descemet's membrane, tearing of the scleral flap, and bleeding from the episcleral layer [Nutan]. Due to these severe complications, safer surgical techniques have been and continue to be explored for use in treating glaucoma.

5.3. Surgical Approaches and Other Conventional Glaucoma Surgeries

There are two different approaches to a trabeculectomy, along with other types of glaucoma surgery: ab interno (from the inside) and ab externo (from the outside). The ab externo approach is considered to be non-penetrating because the surgeon does not enter

or penetrate the anterior chamber. There are conflicting opinions on whether or not non-penetrating surgeries should be considered before penetrating surgeries, but they do have the "advantage of minimizing the risk of postoperative complications related to hypotony" [Lachkar].

A trabeculectomy is considered to be a partial thickness filtering surgery, which is also sometimes referred to as a guarded, protected, or subscleral filtering procedure. During a guarded filtering procedure the filtering sclerostomy, or surgical perforation of the sclera, is protected by partially closing the scleral flap. In contrast, no guard covers the external surface of the sclerostomy in a full thickness procedure. The reduction in IOP is comparable in both partial and full thickness surgeries, but the partial thickness procedures do not have as many complications related to lower pressures due to the presence of the scleral flap [Watkins]. Full thickness procedures are generally avoided due to their severe complications. Two different types of conjunctival flaps can be used to cover the incision in the sclera, with the limbus based conjunctival flap being preferred over the fornix based flap because it creates a more water tight seal [Nutan].

Several other types of glaucoma drainage surgery include trabeculotomy, goniotomy, a few different full thickness procedures, iridotomy, and iridectomy. In addition, several laser surgeries can be used in the treatment of glaucoma. Laser trabeculoplasty and laser cyclophotocoagulation are the main laser treatments for primary open angle glaucoma. Though laser techniques are not typically as effective as conventional surgery in lowering IOP they have fewer complications. Other conventional surgical techniques and laser surgeries are discussed in more detail in Appendix C.

5.4. Nonpenetrating Glaucoma Surgeries and Canaloplasty

Nonpenetrating glaucoma surgeries have recently been utilized because they avoid the severe hypotony that can result from entrance into the anterior chamber. There are three main types of nonpenetrating surgical procedures used for the treatment of glaucoma, all of which are guarded procedures. The first type of nonpenetrating surgery is a deep sclerectomy, the second is an external trabeculectomy, and the third is a viscocanalostomy [Johnson 2001]. Oftentimes the deep sclerectomy is combined with an ab externo trabeculectomy. During a deep sclerectomy a portion of the sclera and cornea near to the trabecular meshwork is removed as in a trabeculectomy, but Descemet's membrane is left intact and Schlemm's canal is unroofed. "In deep sclerectomy the removal of both deep scleral flap and corneal stroma behind the anterior trabeculum and the Descemet membrane allows the aqueous humor to leave the anterior chamber through the intact trabeculodescemetic membrane" [Lachkar]. The inner wall of Schlemm's canal and the adjacent layers of the trabecular meshwork are removed during an external trabeculectomy. The viscocanalostomy procedure is similar to a deep sclerectomy, except a viscoelastic substance is injected into the surgically opened ostia, or ends, of Schlemm's canal [Peckar]. In theory, the viscoelastic substance expands certain areas of Schlemm's canal and the aqueous collector channels to increase outflow.

Comparison between nonpenetrating and penetrating procedures is difficult because of surgical variations. Several previously reported results show that nonpenetrating deep sclerectomy with external trabeculectomy gives a reduction in IOP comparable to a conventional trabeculectomy, but with less complications [Lachkar].

One study showed the success rate (IOP less than 21 mm Hg and no glaucoma medication necessary) at 18 months was 80% in the deep sclerectomy with external trabeculectomy group but 88% in the conventional trabeculectomy group. In this study goniopuncture, sometimes used after a deep sclerectomy with external trabeculectomy, was used as a follow-up procedure in a third of the patients. A different study found a statistically significant difference between the postoperative IOPs of patients who underwent a deep sclerectomy with external trabeculectomy (20.9 mm Hg) and patients who underwent a conventional trabeculectomy (17.3 mm Hg) [Chiselita].

Canaloplasty is also a novel nonpenetrating surgical procedure similar to a viscocanalostomy that can be used for glaucoma therapy. During a canaloplasty, a microcatheter is inserted into Schlemm's canal (see Figure 2.1) and then a viscoelastic substance is continually injected through the catheter as it is slowly removed from the incision [Peckar]. Canaloplasty and viscocanalostomy are newer procedures that have shown some promising results in clinical trials, giving similar reductions in IOP to trabeculectomy with low rates of hypotony and other complications. One study showed that viscocanalostomy had an overall success rate (IOP reduction less than 30% but IOP less than or equal to 20 mm Hg) of 88% at 1 year, 90% at 2 years, and 88% at 3 years [Sunarevic-Mégevand].

Another randomized trial that compared viscocanalostomy and trabeculectomy a year following surgery showed a comparable reduction in IOP in between the viscocanalostomy group (24.6 mm Hg preoperative to 14.0 mm Hg postoperative) and trabeculectomy group (22.3 mm Hg preoperative to 13.3 postoperative) [Carassa]. One

surgeon said 85% of his viscocanalostomy patients and 95% of his canaloplasty patients had an IOP less than 21 mm Hg following surgery [Peckar]. Overall, the nonpenetrating procedures oftentimes give a similar reduction in IOP with fewer complications when compared to a trabeculectomy. Therefore, I believe that nonpenetrating procedures, in particular the seemingly successful canaloplasty procedure, should be considered as a replacement for conventional surgical methods.

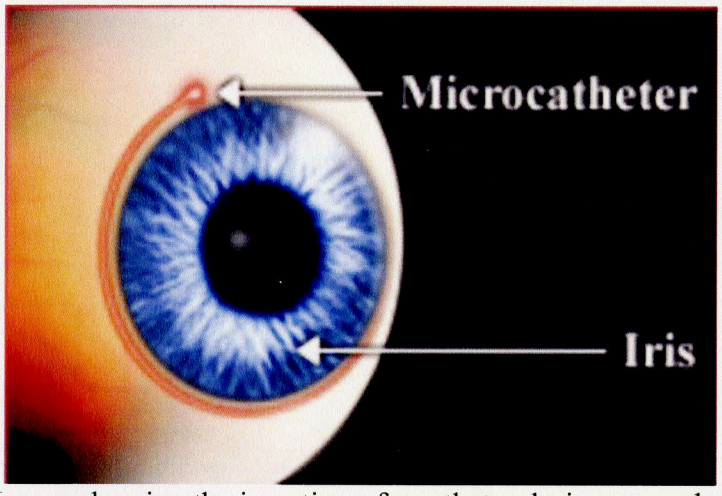

Figure 5.1 Image showing the insertion of a catheter during a canaloplasty [New Glaucoma Treatments]

5.5. Trabectome™

A tool called a Trabectome™ is currently under investigation to evaluate its safety and efficacy for use during drainage surgery. The Trabectome is a microelectrocautery device that was developed in 2004 for the treatment of glaucoma [Mosaed]. An ab interno approach is used with this surgical instrument in order to ablate a portion of the trabecular meshwork and inner wall of Schlemm's canal, opening up a

portion of the drainage channel (see Figure 2.2). The Trabectome has been shown to have the ability to disrupt the desired area of the trabecular meshwork and Schlemm's canal without damaging surrounding tissue [Francis 2006]. With the use of the Trabectome, the conjunctiva, sclera, and Tenon capsule are preserved, allowing for additional drainage surgery if necessary [Liu]. Transient hyphemia, or bleeding into the anterior chamber, is the most common complication seen when the Trabectome is used, with no patients reporting permanent visual impairment, choroidal effusions, or infections [Francis 2006]. In addition, there is no bleb formation with this process, so there is no risk of bleb infection, bleb leak, saucer shaped excavations at the edge of the cornea, or inflammation in the intraocular cavities.

A success rate (defined as no additional glaucoma surgery) of 89.6% has been shown for procedures where the Trabectome is used, with the IOP typically stabilizing in the mid-teens through 5 years of follow-up in patients where the procedure is successful. Even though there is a greater reduction in IOP following a traditional trabeculectomy (12.7 mm Hg postoperative for trabeculectomy versus 16.1 mm Hg postoperative with the Trabectome), there are less complications with the use of a Trabectome, making it a promising new tool for glaucoma therapy [Francis 2006, Liu].

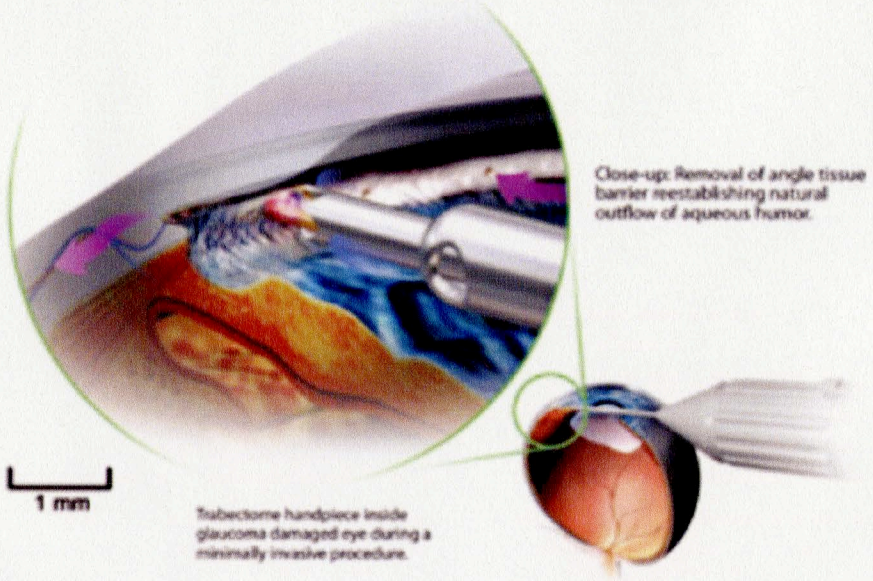

Figure 5.2 Image showing how the Trabectome^{TM} surgical tool is used to remove portions from the trabecular meshwork during glaucoma drainage surgery [Stamper 2011]

5.6. Fugo Blade and Excimer Laser Trabeculotomy

Other new types of surgical therapy include Fugo blade transciliary filtration and Excimer laser trabeculotomy (ELT). "The Fugo blade is an electrosurgical device that produces noncauterizing hemostasis and precise tissue cutting while minimally affecting the adjacent tissue and sterilizing the wall of the incision" [Francis 2011]. Transciliary filtration with a Fugo blade involves creating an opening from the posterior chamber of the eye to the subconjunctival space in order to filter out aqueous humor. Antifibrotic agents are not necessary following the transciliary filtration procedure, and it is a quick, low cost option. However, there is bleb formation and the risk of hypotony after surgery. A retrospective case study on Fugo blade transciliary filtration showed that 85% of

patients had an IOP of less than 21 mm Hg without medications or the need for a repeat surgery. In another study, 76.6% of patients had an IOP less than 30 mm Hg with no need for supplementary drugs after a Fugo blade transciliary filtration [Dow].

A goniotomy, or the creation of an opening through the trabecular meshwork directly to Schlemm's canal, can also be completed ab interno with a Fugo blade. Performing a goniotomy with a Fugo blade has the advantages of no bleb formation, low risk of hypotony, and no destruction of the conjunctiva [Singh 2006]. Disadvantages of the procedure include blockage of the new opening through fibrosis and difficult control of ablation depth. A small study showed that 87.5% of patients had an IOP less than 21 mm Hg 6 months after a Fugo blade goniotomy. More experiments need to be done to study the effectiveness and safety of these new Fugo blade techniques, but the few published studies show that they have a lot of potential.

Excimer laser trabeculotomy (ELT) uses a xenon chloride excimer laser connected to a fiberoptic probe to make holes in the trabecular meshwork and inner wall of Schlemm's canal. Unlike other laser surgeries, the ablation during an ELT is more controlled and the trabecular meshwork does not sustain heat damage. However, the smaller openings created during an ELT are more prone to closure and it is more invasive than other laser surgeries. A small prospective study showed an IOP decrease of 20% with no supplementary medication necessary in 54% of patients [Babighian]. Since bleb-related issues, fibrosis, or channel blockage can cause failure after conventional surgeries, drainage devices and other approaches should be considered to create a permanent outflow pathway.

6. IMAGING METHODS TO VIEW AQUEOUS OUTFLOW

Many of the surgical techniques previously discussed do not protect lymphatic outflow in the eye, so a new surgical approach was explored in a rabbit study. Though trypan blue was the chosen imaging method in this study given our resources and other practical constraints, other imaging methods to visualize lymphatic vessels may be more useful in developing a surgical technique to preserve the lymphatic channels.

6.1. Advantages to Effective Imaging of Aqueous Outflow

"Developments in optical imaging techniques designed for the eye, such as scanning laser polarimetry, confocal scanning laser ophthalmoscopy, and optical coherence tomography, have taken advantage of the naturally clear optical pathway that provides access to the internal features of the eye" but "can acquire only limited information beyond the level of the retinal pigment epithelium" [Townsend]. Discovering a way to clearly and accurately imaging the aqueous outflow pathway would be extremely valuable. Methods to visualize lymphatic vessels near the eye have recently been introduced because lymphatics are now thought to have a large contribution to aqueous humor outflow, particularly when the eye is in a diseased or inflamed state [Yu]. Imaging modalities such as contrast-enhanced ultrasound have been successfully used for imaging lymphatics [Goldberg]. More recently, photoacoustic imaging has been used for viewing the vasculature around the eye [Li].

Previous experiments provide good evidence that imaging the outflow pathways in the eye with greater accuracy and fidelity is possible. It is extremely important to be

able to visualize the outflow pathways in the eye both before and after conventional surgery, laser surgery, or implantation of a glaucoma shunt in order to improve upon the technique or device. Also, a great deal currently not understood about the outflow pathways in the eye could be elucidated with further imaging studies. There are many different imaging methods that have the potential to accurately image the aqueous humor outflow pathways in the eye. All of these imaging methods need to be compared to ensure fidelity in imaging both the lymphatic channels and vasculature near the eye.

6.2. Fluorescein Imaging

Fluorescein is a substance that can be used as a tracer in the imaging of the lymphatic networks. Fluorescein has an excitation wavelength of 465-490 nm, and the camera should have a 520-530 nm barrier filter to properly capture images. Typically, fluorescein is used in fluorescein angiography, which is "an important tool for ophthalmologists in understanding, diagnosis, and treatment of retinal disorders" [Bennett]. When injected into systemic circulation it can be used to visualize the vasculature. However, studies have also shown that fluorescein can be used to trace and study aqueous humor outflow (see Figure 6.1) [Yu]. Though using a pre-placed contrast agent to visualize and protect the lymphatics during surgery would be beneficial, tests have shown that several of the dyes commonly used in the eye, such as indocyanine green and trypan blue, are toxic to retinal cells [Kodjikian]. Therefore, care must be taken when using these contrast agents in the eye, and less toxic alternatives should be considered.

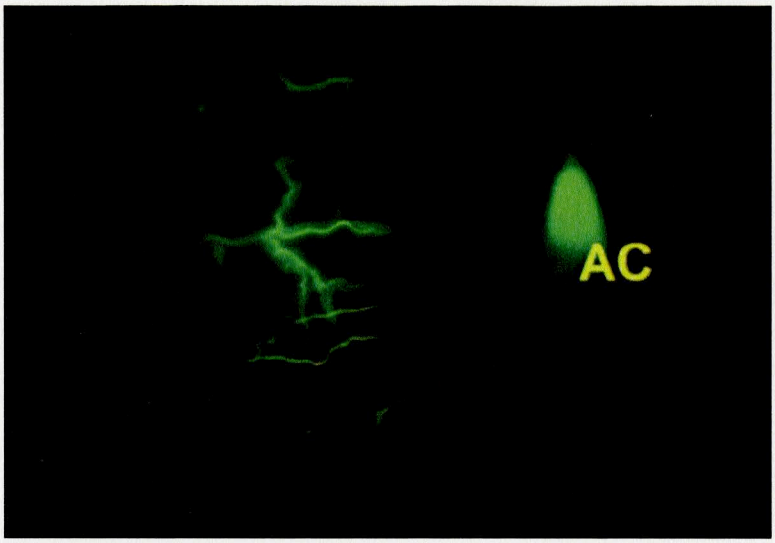

Figure 6.1 Fluorescein imaging of the aqueous humor outflow pathway in the eye, following injection into the anterior chamber of a rabbit eye [Yu]

6.3. Preliminary Imaging Study with Fluorescein

Imaging studies were completed to determine the most effective way to visualize aqueous humor outflow. The studies were performed on enucleated eyes with IACUC approval, under bioproducts protocol #10-236. Porcine, bovine, and ovine eyes were procured from the University of Arizona meat science lab.

Prior to doing any perfusion studies, several images were taken with the Zeiss slit lamp microscope in order to visualize the blood vessels. With 16x and 40x magnification, the vessels in the enucleated eyes were clearly visible. The amount of light was adjusted to allow for more clear images of the vessels.

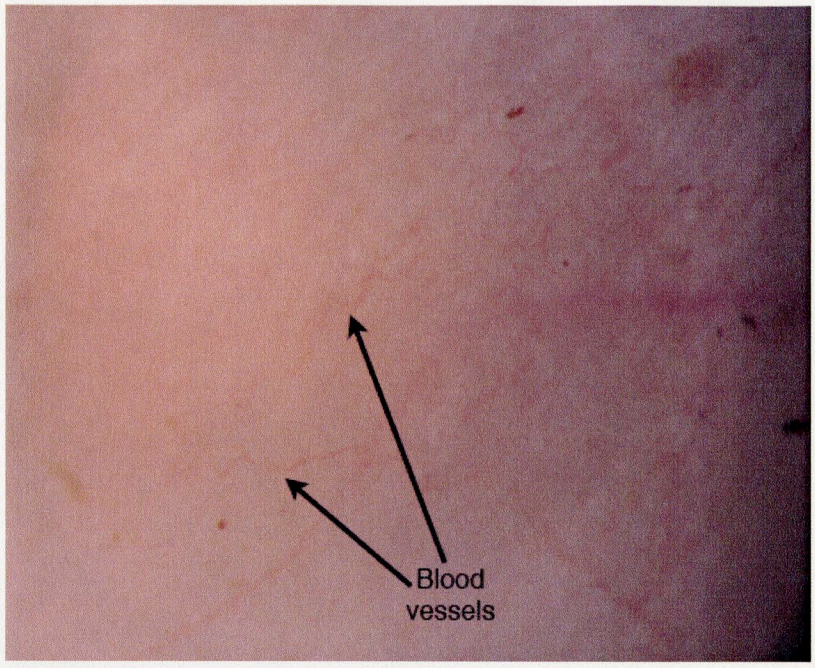

Figure 6.2 Image showing scleral blood vessels in an ovine eye

Fluorescein was used in the attempt to visualize the aqueous humor outflow in the eye. In order to mix the fluorescein, sodium ophthalmic strips (purchased from Bio Glo, 1 mg strips) were placed in 2-3 mL of balanced salt solution (purchased from Akorn) to make a solution of sufficient concentration to be clearly visible. Images were taken from a Zeiss slit lamp microscope with up to 40x magnification on either a standard Canon rebel digital camera attached to the slit lamp microscope, or a 10.1 megapixel Nikon coolpix digital camera through the oculars. At times, a cobalt filter was used in order to filter out certain wavelengths of light and selectively excite fluorescein. The cobalt filter allowed for better visualization of the fluorescein alone.

Several different methods were used in order to view perfusion in the eye. First, the perfusion of a one-time injection of dye directly into the anterior chamber was

examined as a preliminary experiment. Then, the diffusion of the dye from a subconjunctival bleb was observed. Lastly, the continuous perfusion of dye into both the anterior chamber and subconjunctival bleb was viewed at a constant pressure. The setup for the continuous perfusion study was a syringe connected to a tube with a 23 gauge needle at the end. The 23 gauge needle was then inserted into either the anterior chamber or subconjunctival space of the eye (see Figure 6.5 for the experimental setup). The hydrostatic pressure of the dye being injected into the enucleated eyes was controlled by adjusting the height of the syringe to the desired value of pressure head. The pressure was typically set close to physiological pressure, around 20 mm Hg.

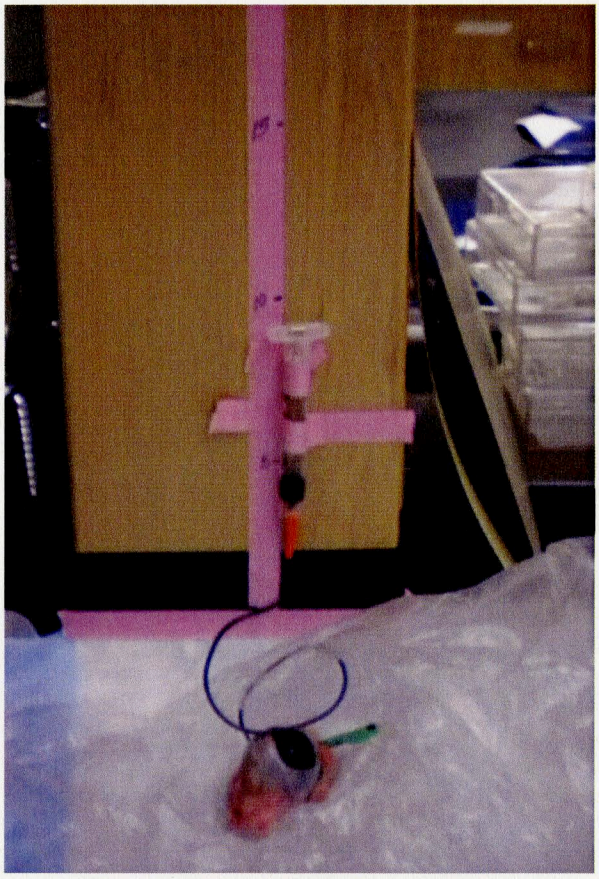

Figure 6.3 Setup of the continuous perfusion pressure studies

The fluorescein was visible in the anterior chamber of many of the samples after injection, but the dye was not typically visible outside of the anterior chamber or the subconjunctival blebs (Figures 6.4 and 6.5). The subconjunctival blebs became larger over time, showing that the fluorescein did diffuse through the subconjunctival space (Figure 6.6). Neither the aqueous collector blood vessels nor potential lymphatic collector vessels were highlighted by the fluorescein dye in the eyes that were tested. Even the addition of the cobalt filter did not seem to assist with visualization of the dye flowing through the vessels. In only one case was there a blood vessel visibly filled with fluorescein (Figure 6.10 to Figure 6.12).

A potential explanation for not seeing the fluorescein is that the concentration of the fluorescein dye, though enough to be visible to the dye prior to injection, may not have been high enough to be visible through tissue. Another possible explanation for failure in some of the samples is that removal of the eyes from the animals could impair the function of the vessels and outflow network, particularly the lymphatic vessels, to the point where perfusion outside of the anterior chamber does not occur. One thing that may have helped with the fluorescein imaging is to have a filter on the camera itself to selectively view the wavelength of light that fluorescein gives off when excited by light from the cobalt filter. Fluorescein images are shown from Figure 6.4 to Figure 6.12.

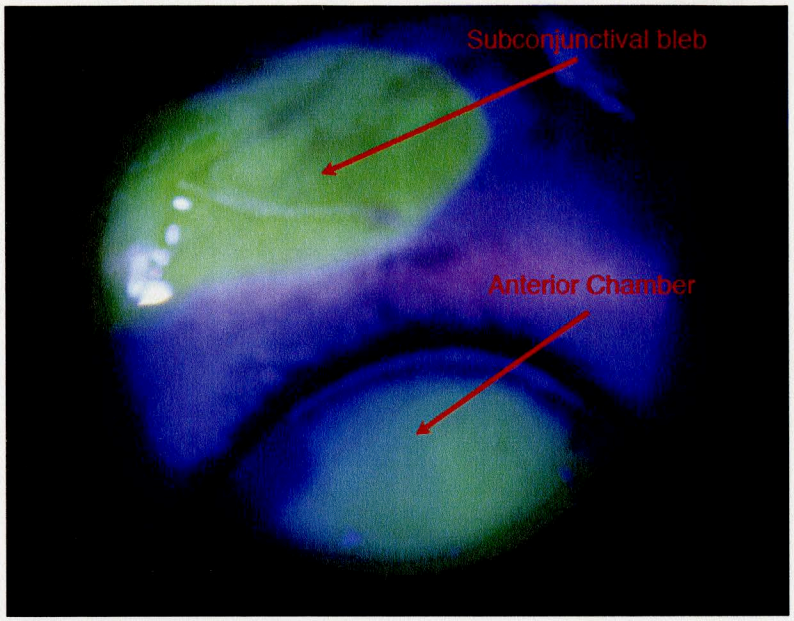

Figure 6.4 Image showing fluorescein dye in the anterior chamber and a conjunctival bleb filled with fluorescein dye in a bovine eye (10x magnification)

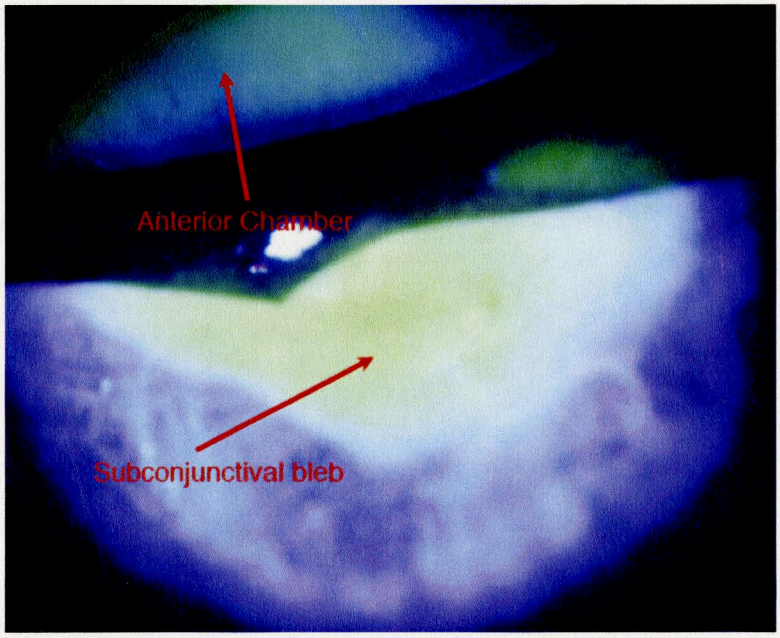

Figure 6.5 Image showing fluorescein dye in the anterior chamber and a conjunctival bleb filled with fluorescein dye in a bovine eye (16x magnification)

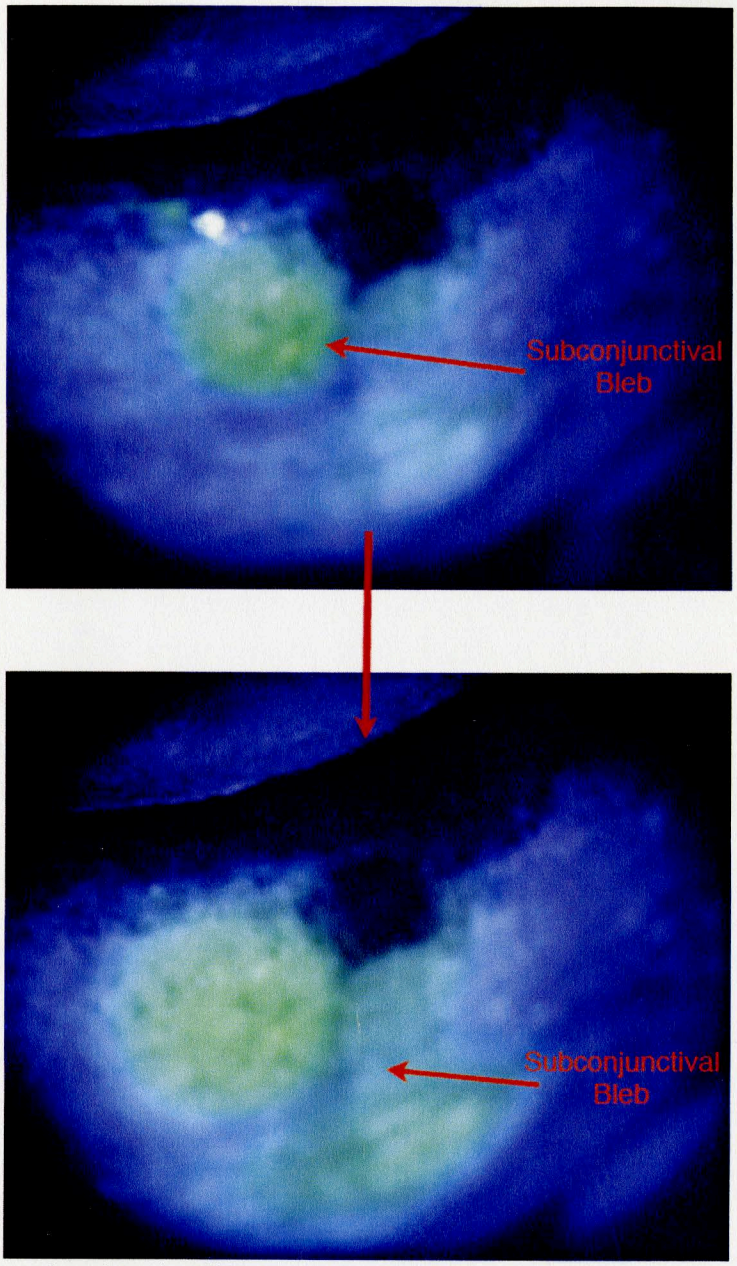

Figure 6.6 Image showing the diffusion of a subconjunctival bleb of fluorescein through the tissue as it grows in size over time from the top picture to the bottom picture in a bovine eye (16x magnification)

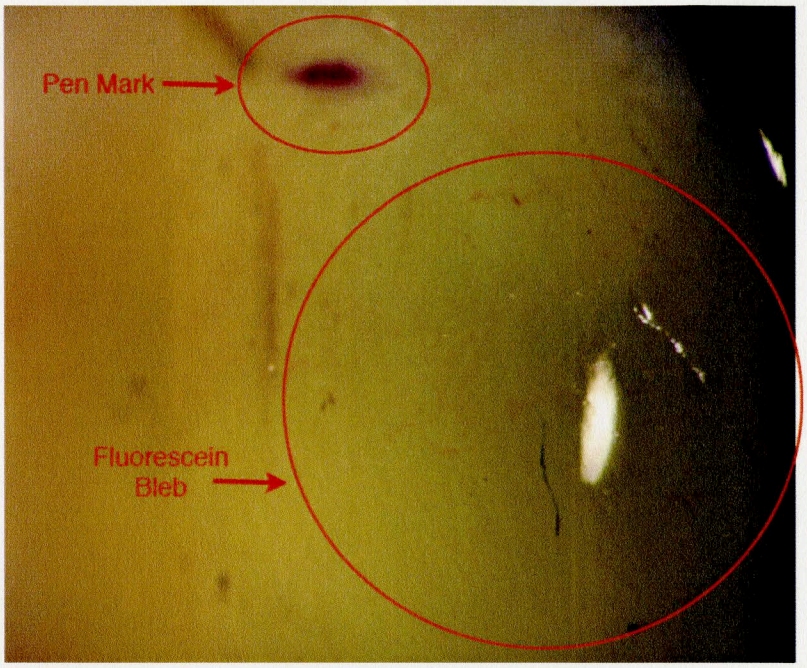

Figure 6.7 Image showing a subconjunctival bleb of fluorescein in a porcine eye (25x magnification)

Figure 6.8 Image showing a subconjunctival bleb of fluorescein in a porcine eye (same as in Figure 6.7), under a cobalt filter (6x magnification)

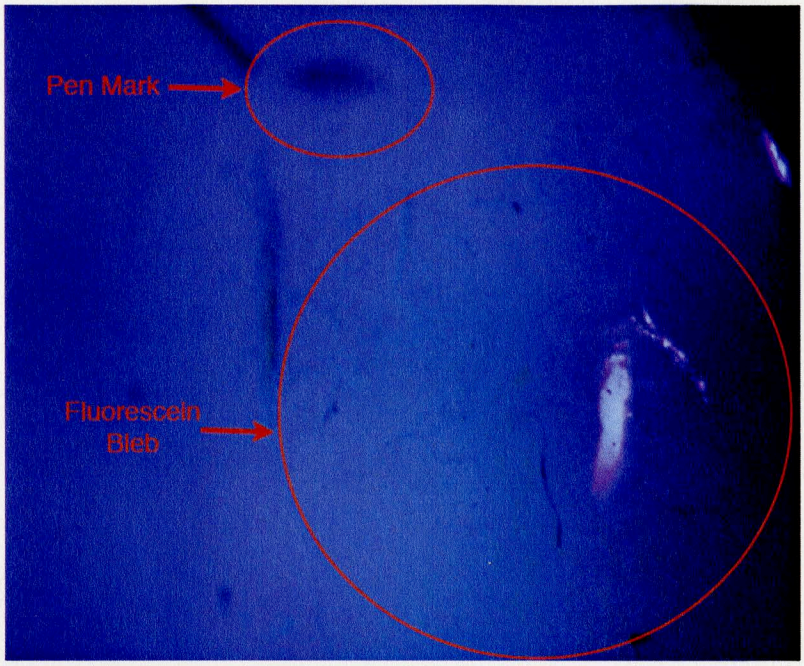

Figure 6.9 Image showing a subconjunctival bleb of fluorescein (same as in Figure 6.7) under a cobalt filter in a porcine eye (25x magnification)

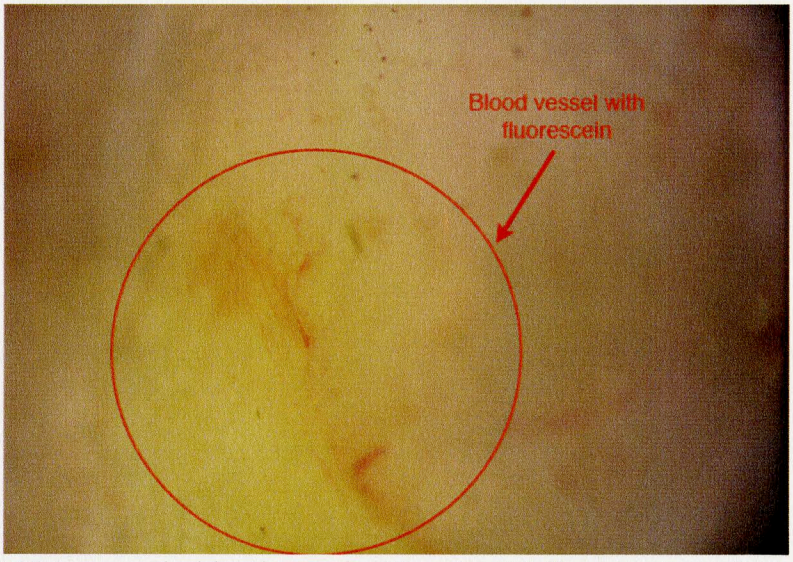

Figure 6.10 Image of a blood vessel with potential fluorescein perfusion, near a subconjunctival bleb of fluorescein in a porcine eye (25x magnification)

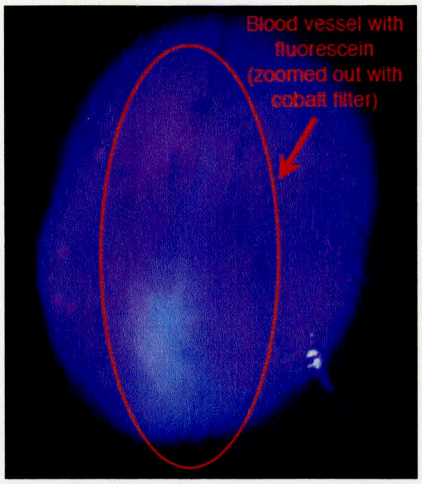

Figure 6.11 Image of a blood vessel with potential fluorescein perfusion, near a subconjunctival bleb of fluorescein (same as in Figure 6.10) and under a cobalt filter in a porcine eye (6x magnification)

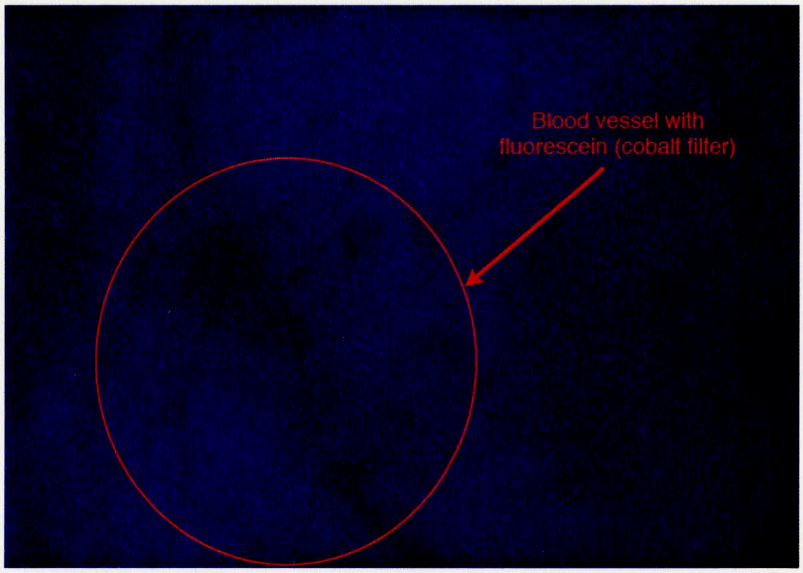

Figure 6.12 Image of a blood vessel with potential fluorescein perfusion, near a subconjunctival bleb of fluorescein (same as in Figure 6.10) and under a cobalt filter in a porcine eye (25x magnification)

6.4. Trypan Blue Imaging

Trypan blue is a vital dye, and it is commonly used in microscopy to assess the viability of different cells. Though trypan blue is most commonly used in ophthalmology

to view the lens capsule during cataract surgery, it has also been shown to be effective in visualizing the lymphatic networks in the eye [Jacobs]. Trypan blue can be injected into the subconjunctival tissue to create interstitial tissue fluid, which then drains through the lymphatics (see Figure 6.13). The subconjunctival pool of trypan blue is observed in order to evaluate if it remains stagnant, meaning lack of lymphatic drainage, or if it spreads away from the blister, indicating the existence of lymphatic drainage and showing the distinct branch-like tributaries [Yu]. Lymph dynamics and principal collectors in all four quadrants can be recorded by slit lamp microscopy and digital video microscopy. During studies with Fugo blade transciliary filtration, Singh et. al learned more about the lymphatic drainage system by using trypan blue. "Using trypan blue staining, Dr. Singh was able to visualize the lymphatic drainage system as it carried the aqueous away from the ciliary body" [Bethke].

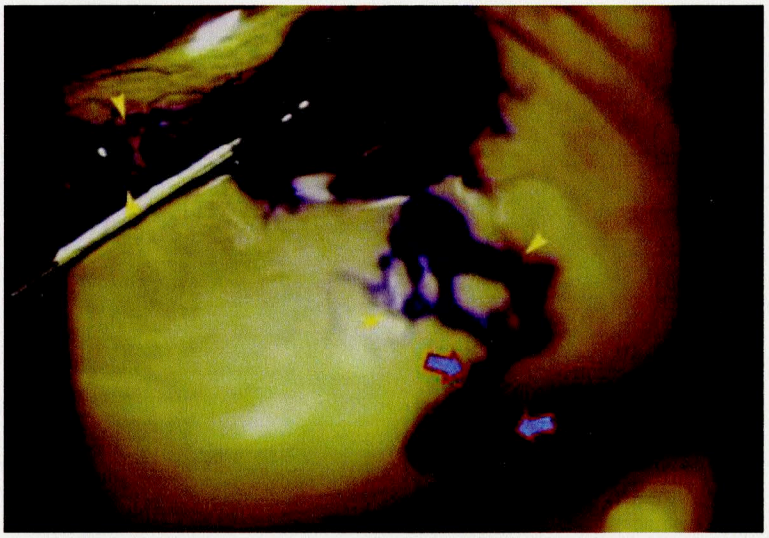

Figure 6.13 Trypan blue perfusion into the lymphatic networks of a rabbit eye following injection into a subconjunctival bleb [Yu]

6.5. Preliminary Imaging Study with Trypan Blue

In our study, when the fluorescein imaging did not properly show the path of the aqueous humor as it left the anterior chamber or a subconjunctival bleb, trypan blue (purchased from Sigma-Aldrich) imaging was also tested. The same setup was used in the fluorescein study and the trypan blue study. A benefit of using trypan blue over fluorescein is that it can be visualized without the use of any filters. The trypan blue imaging much more clearly showed the aqueous humor outflow, especially when there was continuous perfusion of the dye into either the anterior chamber or a subconjunctival bleb at a known pressure (see Figures 6.14 to 6.17). Several of the vessels visible with the perfusion of trypan blue resembled lymphatics when compared to pictures in previous studies by Yu et. al and Singh et. al (see Figure 6.15). The vessels showed possible valve structures and were distinct from nearby blood vessels, both things characteristic of lymphatic vessels (see Figures 6.14 to 6.16). Trypan blue images are shown from Figure 6.14 to Figure 6.18.

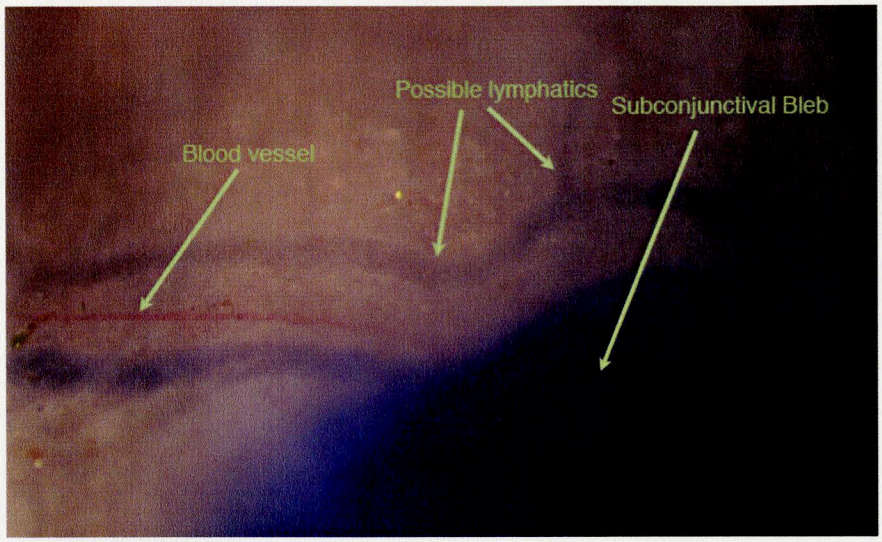

Figure 6.14 Image showing trypan blue perfusion from a subconjunctival bleb to potential lymphatics, near to a blood vessel in an ovine eye (40x magnification)

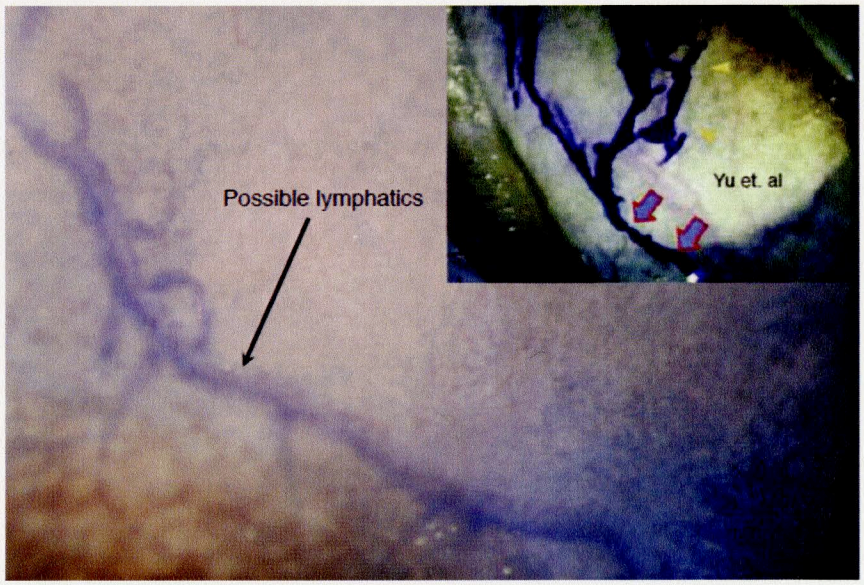

Figure 6.15 Image of potential lymphatics perfused with trypan blue in an ovine eye (40x magnification) compared with an image of lymphatics from Yu et. al

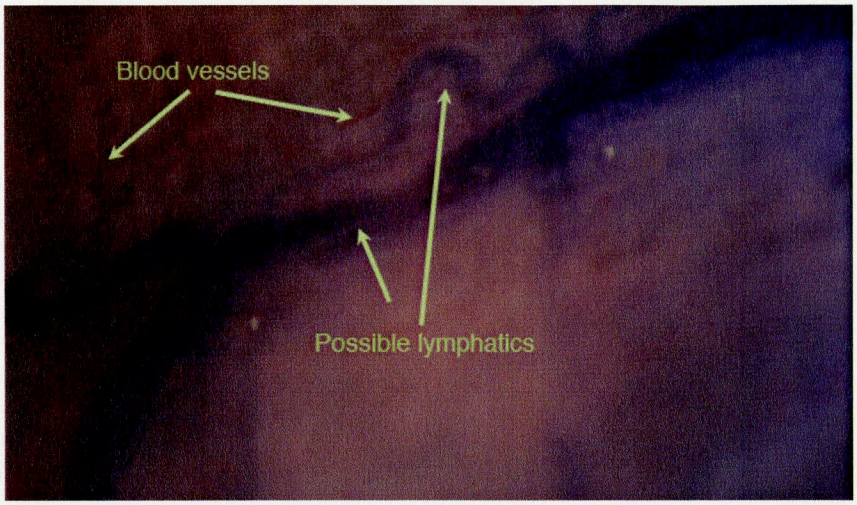

Figure 6.16 Image showing trypan blue perfusion from the anterior chamber to potential lymphatics, near to blood vessels in an ovine eye (40x magnification)

Figure 6.17 Image showing trypan blue perfusion from the anterior chamber to potential lymphatics in an ovine eye (40x magnification)

A study performed with the constant injection of trypan blue at different hydrostatic pressures showed that perfusion from the anterior chamber into the nearby vessels occurs at around 15 mm Hg, which is close to the physiological pressure in the

eye (Figure 6.18). No perfusion was seen into vessels near to the anterior chamber at 5 mm Hg and 10 mm Hg. However, perfusion began at 15 mm Hg, and dye was seen in even more vessels as the pressure was raised to 20 mm Hg.

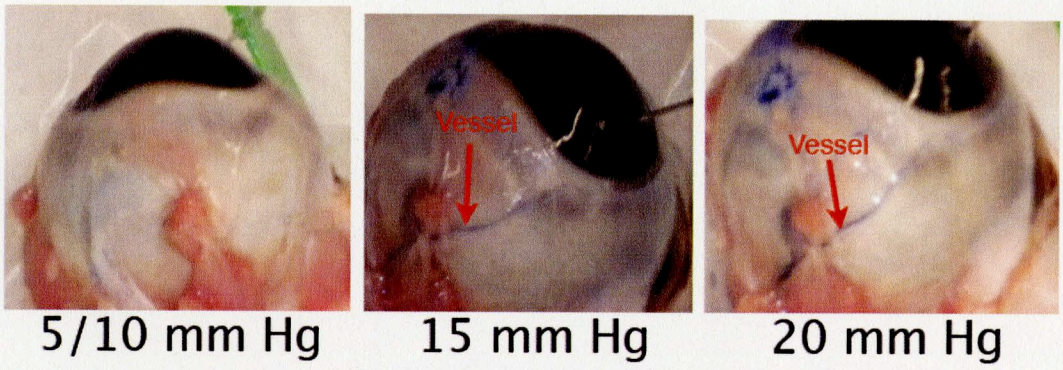

Figure 6.18 Image showing the perfusion of trypan at different hydrostatic pressure values, from 5 mm Hg to 20 mm Hg, in an ovine eye

Overall, the imaging studies showed that trypan blue is a more convenient and effective method of imaging aqueous humor outflow when compared with fluorescein imaging. Vessels that resembled lymphatic channels were highlighted in enucleated eyes using trypan blue. In addition, perfusion performed at different pressures showed evidence that the trypan blue did not fill the vessels until the hydrostatic pressure was 15 mm Hg. In order to confirm that the lymphatics can be viewed using trypan blue, more *in vivo* studies should also be completed.

6.6. Two-Photon Microscopy

Fluorescent dyes could also be used as contrast agents for *in vivo* two-photon fluorescent light microscopy [So]. "Two-photon microscopy is a nonlinear imaging

technology with subcellular resolution capabilities that has been previously used to image ocular tissues without the need for fixation" [Johnson 2011]. There are many different fluorescent proteins that could be used for imaging, including green fluorescent protein, yellow fluorescent protein, and cyan fluorescent protein. In order to prevent excessive diffusion outside of the lymphatic networks in the eye, the fluorescent molecules could potentially be coupled to larger molecules such as dextran. Some molecules in the body are naturally fluorescent, such as NADH and NADPH, and therefore different cellular processes can be monitored using two-photon excitation autofluorescence microscopy [Imanishi].

Second harmonic generation is another two-photon technique that occurs when the structure being observed scatters the two infrared photons instead of absorbing them, and the photons coalesce into one higher energy photon. Two-photon microscopy has been used to study the action of the cells in the retina and the structure of the cornea, and it has aided understanding of these tissues. A limitation of two-photon microscopy is its depth of penetration. One study looking at the diffusion of a tracer through the sclera showed that imaging with two-photon microscopy was possible to a depth of 340 micrometers, the average thickness of the human sclera [Kek]. Another recent study attempted to image the aqueous outflow pathways in a mouse eye (Figure 6.19). This study showed that "two-photon microscopy may be used for noninvasively imaging the conventional aqueous outflow pathway in mouse models of glaucoma," with expected future work on live animals [Johnson 2011]. Two-photon microscopy is a fairly new

imaging technique that has potential for imaging the aqueous humor outflow pathway *in vivo*, though it probably lacks the ability to distinguish specific vasculature.

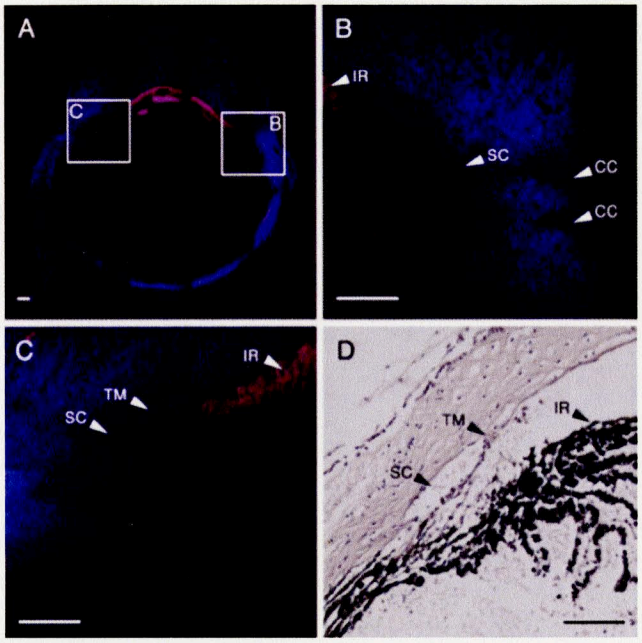

Figure 6.19 Image showing a comparison of auto-fluorescent two-photon microscopy and histology slides [Johnson 2011]

6.7. Ultrasound

Sonographic imaging could be used to visualize the vascular and lymphatic networks in the eye *in vivo*. In previous studies, lymphatic channels and sentinel lymph nodes have been successfully imaged using microbubble contrast agents, despite their size limitations (Figure 6.20, top) [Goldberg, Bloch]. Various contrast agents need to be utilized in order to gauge whether or not they can effectively image the lymphatics near the eye. These ultrasound contrast agents could be monitored for uptake into the lymphatic channels as described previously using fundamental 2-D gray scale and color

flow imaging (CFI) as well as gray scale pulse inversion harmonic imaging (GSPIHI) and multiplanar 3-D sonography [Goldberg].

Sonazoid, Definity, and Optison are three potential microbubble contrast agents that could be tested for efficacy in highlighting the lymphatic and vascular pathways in the eye. In addition, the blood flow near the eye can be viewed using doppler color flow imaging. One study looked at using volumetric color doppler imaging in order to image ocular blood flow in glaucoma patients [Zeitz]. The study showed that regular color doppler imaging gave very reproducible results for blood velocity in the opthalmic artery and central retinal artery, while volumetric blood flow was not as accurate and needs to be optimized before its used in a clinical setting (Figure 6.20, bottom).

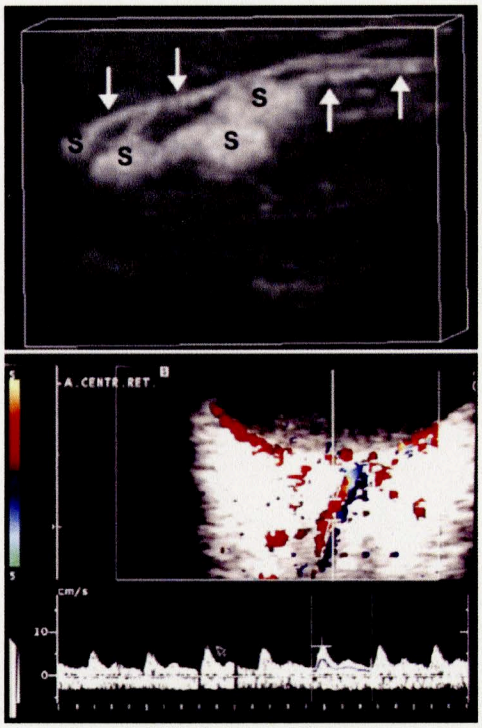

Figure 6.20 Image showing the highlighting of lymphatic vessels using an ultrasound contrast agent (top) and the flow of blood in ocular blood vessels using color doppler imaging (bottom) [Goldberg, Zeitz]

6.8. Magnetic Resonance Imaging (MRI)

MRIs are routinely done on the eye to look for things such as tumors, infections, ocular neuropathy, or swelling of the optic nerve [Berkowitz]. MRI is an imaging technique that uses varying magnetic fields to distinguish between different types of tissue. One study attempted to use diffusion MRI to distinguish between different ocular conditions [Xu]. Though there are difficulties involved with finding the MRI sequence that gives the best contrast and most accurate results to make a diagnosis, researchers are steadily working toward methods that can elucidate different disease states in the optic nerve. A recent study displayed a novel method for diffusion tensor imaging that was able to distinguish patients that were suffering from glaucoma with 94% accuracy [Engelhorn]. Several other studies proved that MRI could be useful for the diagnosis of glaucoma and distinguish between the patients with glaucoma and the healthy controls using parameters such as optic nerve diameter and optic chiasm [Townsend].

MRI can also be used after the implantation of a glaucoma drainage device to visualize its position and function. A study looking at glaucoma shunts showed that "magnetic resonance imaging provides insights into the mechanism of aqueous outflow and causes of failure of shunts" [Detorakis]. Functional MRI (fMRI) is another form of MRI that has been used to map the activity of the retina. In studies with glaucoma fMRI has demonstrated the ability to view "alterations involving the human visual cortex that are consistent with clinically documented loss of visual function" [Garaci].

Another study attempted to visualize ocular blood flow in a rat model of glaucoma using Gd-DTPA contrast-enhanced MRI (Figure 6.21) [Chan]. After systemic

administration of Gd-DTPA Chan et. al noticed an increased signal intensity in the vitreous compartment of the glaucomatous eye but not in vitreous compartment of the normal eye, showing that increased permeability of the blood-aqueous or aqueous-vitreous barrier is a potential step in the progression to glaucoma. Overall, this study showed that imaging of aqueous humor is possible given a contrast agent such as Gd-DTPA, but more experiments need to be completed to assess the possibility of using MRI to visualize outflow through the trabecular meshwork when contrast agent is injected directly into the anterior chamber. An issue with using MRI to properly diagnose glaucoma is that it is not very cost effective compared to the traditional methods for diagnosis [Townsend]. In addition, MRI may not be practical for visualizing aqueous humor outflow in real time due to the long scanning periods.

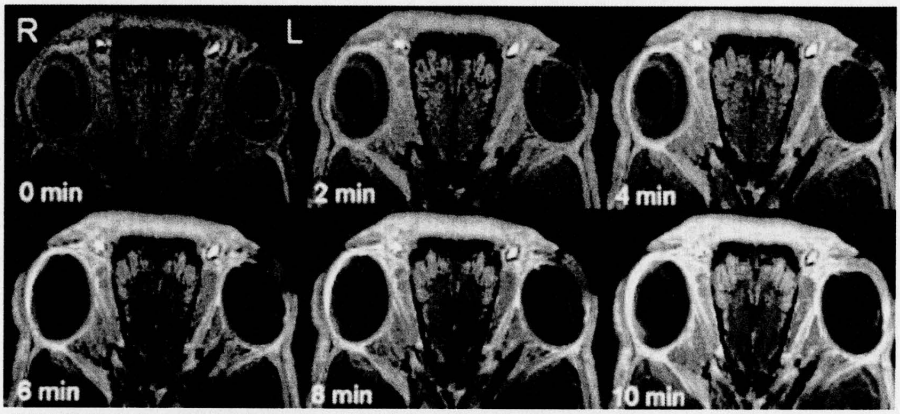

Figure 6.21 MRI image taken at different time points after the injection of Gd-DTPA contrast agent in order to visualize ocular blood flow [Chan]

6.9. Computed Tomography (CT)

Computed tomography (CT) is another imaging method that could potentially be used to image aqueous humor outflow. CT scans compile a series of x-rays to form a 3D

image of the anatomy, with different tissue types being separated by how much they attenuate the x-rays before they are detected. One study that looked at aqueous humor outflow with CT showed that "3D micro-CT can be used effectively for the non-invasive examination of the trabecular meshwork, Schlemm's canal, collector channels and intrascleral vasculature that comprise the distal outflow pathway" (Figure 6.22) [Hann]. During the study several CT contrast agents, including osmium, were also explored. The experiment was completed in enucleated eyes, so the efficacy of this imaging method needs to be tested *in vivo* to prove its usefulness in imaging aqueous humor outflow though the scanning times may prevent real time imaging of aqueous outflow.

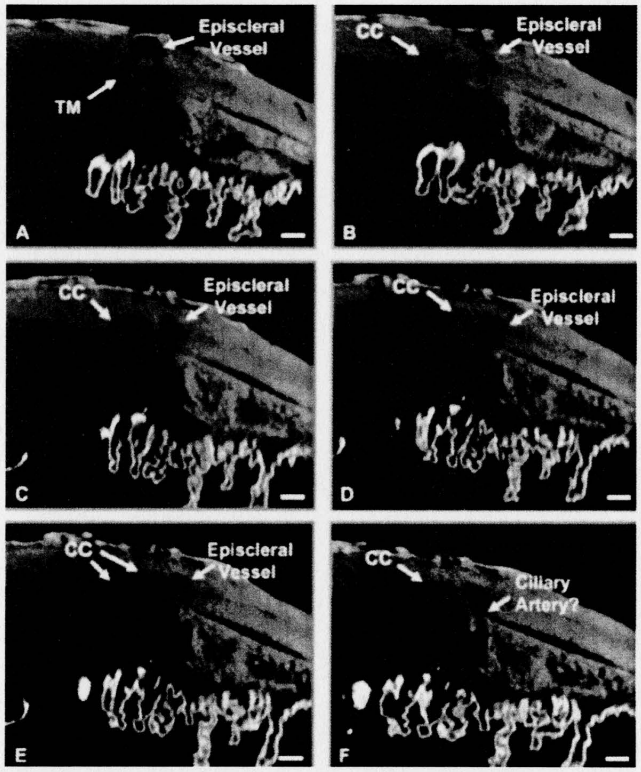

Figure 6.22 CT image of the angle of the eye showing the various structures of the aqueous humor outflow pathway, including vessels and collector channels (CC) [Hann]

6.10. Photoacoustic Imaging and Optical Coherence Tomography

Photoacoustic imaging has recently been used in conjunction with optical coherence tomography to image the microvasculature in the eye [Li]. Photoacoustic imaging involves analyzing the sound waves generated when tissue is heated with a laser, which vary depending on the tissue type. A contrast agent is not necessary to view vasculature due to the optical properties of blood, but it could be useful in distinguishing between the vasculature and lymphatic vessels near the eye. Photoacoustic imaging an extremely promising option for visualizing aqueous humor outflow in the eye, and it has already been used in conjunction with optical coherence tomography to image the blood vessels of the eye (Figure 6.23) [Li]. The photoacoustic images clearly show the ocular blood vessels with remarkable precision, with the overlaid OCT images providing anatomical information. A contrast agent could be injected into the anterior chamber and its outflow could be monitored real time with this imaging modality. In addition, the vessels highlighted with the photoacoustic contrast agent could easily be compared with the vasculature visible in the photoacoustic images and the anatomical information seen in the OCT images.

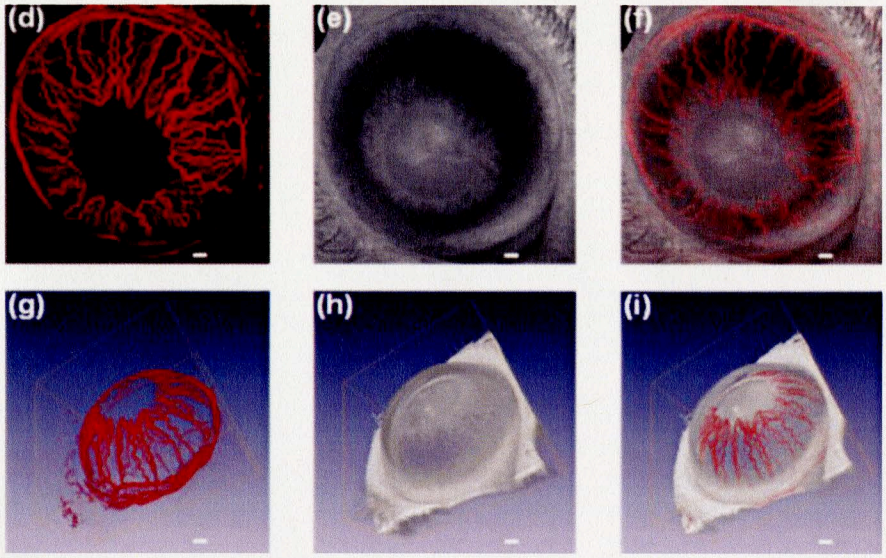

Figure 6.23 Photoacoustic imaging (d and g) combined with OCT imaging (f and i) to visualize both the vasculature and the anatomical structures simultaneously [Li]

Optical coherence tomography (OCT) is another imaging modality that is commonly used to image the eye [Bennett]. The principles behind optical coherence tomography involve imaging the reflection of light from tissue, and since it is based on light it has a very good resolution but not good tissue depth. It can "provide direct cross-sectional images of the macula, retinal nerve fibre layer and optic nerve for objective measurement and clinical evaluation of retinal diseases and glaucoma" [Bennett]. Ocular structures can be very clearly viewed using OCT (Figure 6.24, left), but it is an anatomical imaging modality and would most likely not be practical for following the aqueous humor itself. OCT has recently been used to view the aqueous humor pathways *in situ*. In a recent study, spectral-domain OCT was successfully used to view the aqueous humor pathway, including the aqueous collector veins (Figure 6.24, right). "Schlemm's canal, collector channels, the deep and intrascleral venous plexus, and

episcleral veins were observed throughout the limbus while the aqueous veins could be observed extending into the episcleral veins" [Kagemann].

Overall, I strongly suspect that the combination of photoacoustic and OCT imaging has the most promise for accurately and effectively visualizing the lymphatic networks of the eye *in vivo.* The photoacoustic imaging offers amazing precision in viewing blood vessels, while the OCT offers a great way to view the anatomical structures in the eye. Photoacoustic imaging could easily be adapted to view lymphatic vessels with great exactitude. Also, ultrasound equipment is already available in many hospitals and OCT is available for use in ophthalmology, so these imaging modalities could be used in the clinic prior to glaucoma drainage surgery to visualize and subsequently avoid destroying the lymphatic channels. Future experiments should be completed to confirm the utility of these imaging methods for visualizing aqueous outflow.

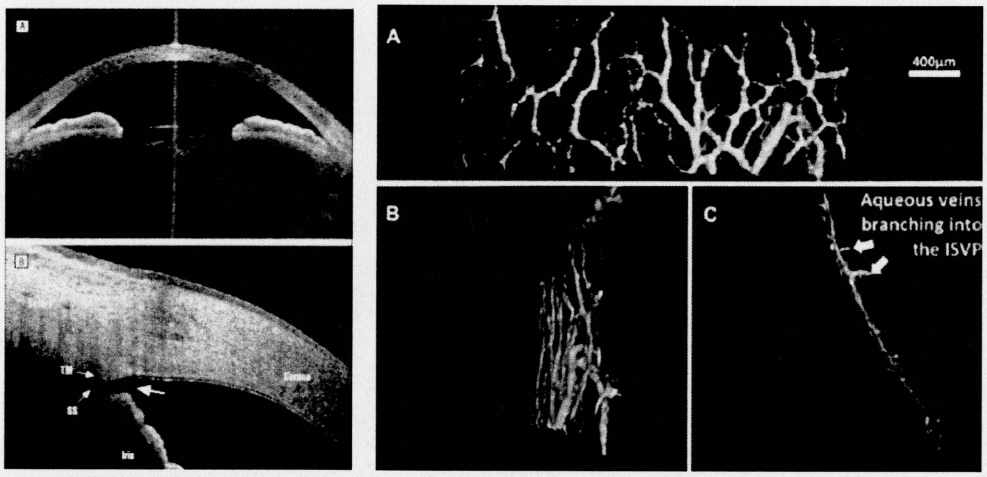

Figure 6.24 OCT image of the anterior chamber, showing the anatomical structures (left), and OCT images showing aqueous humor filling the surrounding veins (right) [Wang, Kagemann]

7. CONCLUSION

There are many different medications used in the treatment of glaucoma, but they have adverse side effects, poor patient adherence, and high costs. When pharmaceutical agents do not sufficiently lower IOP, surgery should be considered. There are a great deal of adverse side effects associated with glaucoma drainage surgery, and many times patients still need to be on pharmaceutical therapy following surgery. Despite these downfalls to glaucoma surgery, an improved surgical treatment as first-line therapy would be extremely beneficial to those suffering from glaucoma who do not have access to the conventional drug treatments. Though traditional glaucoma surgery is still used more frequently than glaucoma shunts, there is a growing trend towards the use of drainage devices. One glaucoma drainage device that shows a lot of promise is the porous e-PTFE device. In preliminary studies the e-PTFE shunt was shown to allow a high enough flow rate to properly lower IOP, even under increased protein concentrations. The e-PTFE implant could even be further modified to optimize its flow properties by changing the pore size, altering the geometry, or attaching molecules to the surface (i.e. antifibrotics).

Recent experiments have shown that lymphatics are essential in aqueous humor outflow following glaucoma drainage surgery. The *in vivo* rabbit experiment showed more evidence of the involvement of lymphatic vessels in aqueous humor outflow following surgery, though more specific histological staining would help validate the presence of lymphatic channels. Further experiments need to be done in order to conclusively elucidate the difference between surgery with a vertical incision and surgery

with a horizontal incision. The original experiments discussed in this paper describe several favorable properties of the e-PTFE glaucoma shunt along with the perfusion of dye through lymphatic and blood vessels in the eye. Overall, previous studies along with the studies conducted in this paper support the hypothesis that e-PTFE and the surgical approach should specifically aim to preserve conjunctival and peribulbar lymphatic and blood vessels as potential conduits for the egress of fluid released by the glaucoma drainage device.

8. FUTURE WORK

In future work, researchers could look into inducing the growth of more vascular and lymphatic vessels near the glaucoma drainage devices in order to improve the outflow pathways of the eye. Various ways to manipulate blood vessel and lymphatic growth near the cornea using factors such as VEGF, VEGF-C and bFGF have been explored in previous experiments [Oh, Chung]. Typically, a reservoir will develop that inhibits outflow and decreases the effectiveness of the shunt. It is thought that this reservoir can create a backpressure that prevents proper aqueous humor outflow. Therefore, it is important to look into methods of reducing this reservoir. Promoting vascular and lymphatic growth near the promising e-PTFE shunt could assist in the reabsorption of fluid, thereby allowing more efficient humor outflow through the device.

Growth factors such as VEGF-A and bFGF can stimulate angiogenesis. Neovascularization and growth factors play an important role in the eye, and anti-VEGF factors are used to treat ocular diseases such as age-related macular degeneration. VEGF-A is a potent angiogenic factor that encourages angiogenesis by increasing cell migration of endothelial cells, proliferation of endothelial cells, matrix metalloproteinase activity, anti-apoptotic activity, and alpha-v beta-3 integrin activity while creating blood vessel lumen and fenestrations [NIH]. This growth factor is also a vasodilator and can increase microvascular permeability. Additionally, it has recently been shown that transgenic overexpression of VEGF-A in mice can also promote lymphatic growth near a wound site [Hong]. Another important angiogenic ligand is bFGF. The bFGF mediates the formation of new blood vessels once it is activated by the degradation of heparin

sulfate in the extracellular matrix during wound healing. In general, bFGF promotes new blood vessel growth by increasing mitogenicity of cells, enhancing precursor endothelial (angioblast) cell growth, and promoting the migration of endothelial cells [Skjerpen]. Also, through previous studies bFGF has been shown to encourage the growth of lymphatic vasculature in the cornea [Chung].

Lymphatic vessel growth is also expected to aid in the drainage of aqueous humor from the e-PTFE glaucoma shunt. In the eye, tissue resembling lymphatic tissue in both structure and function is present in the inner wall of the Schlemm's canal, which is the path that the aqueous humor travels before entering systemic circulation [Ramos]. Lymphatic tissue naturally grows in parallel to the blood vasculature. Lymphangiogenesis is thought to occur when lymphatic sacs develop near veins, as stated by the centrifugal sprouting theory. Therefore, the intraocular pressure (IOP) is controlled by the presence of both blood and lymphatic vasculature. VEGF-C is a growth factor that induces lymphangiogenesis by preferentially binding to the VEGFR-3 receptor, which is essential in lymphatic system development [NIH]. Angiopoietin 1&2 are growth factors typically associated with angiogenesis, but they have also been shown through experimentation to be extremely important in the formation of lymphatic networks [Morisada]. Therefore, both VEGF-C and Angiopoietin 1&2 could be utilized as lymphatic growth factors. In conclusion, enhancing vascular and lymphatic growth while preserving the lymphatic networks already in place is a promising way of improving aqueous outflow from glaucoma drainage devices, of which the e-PTFE implant is an exciting new option.

APPENDICES

Appendix A: Miscellaneous

A.1. Structure of the Eye

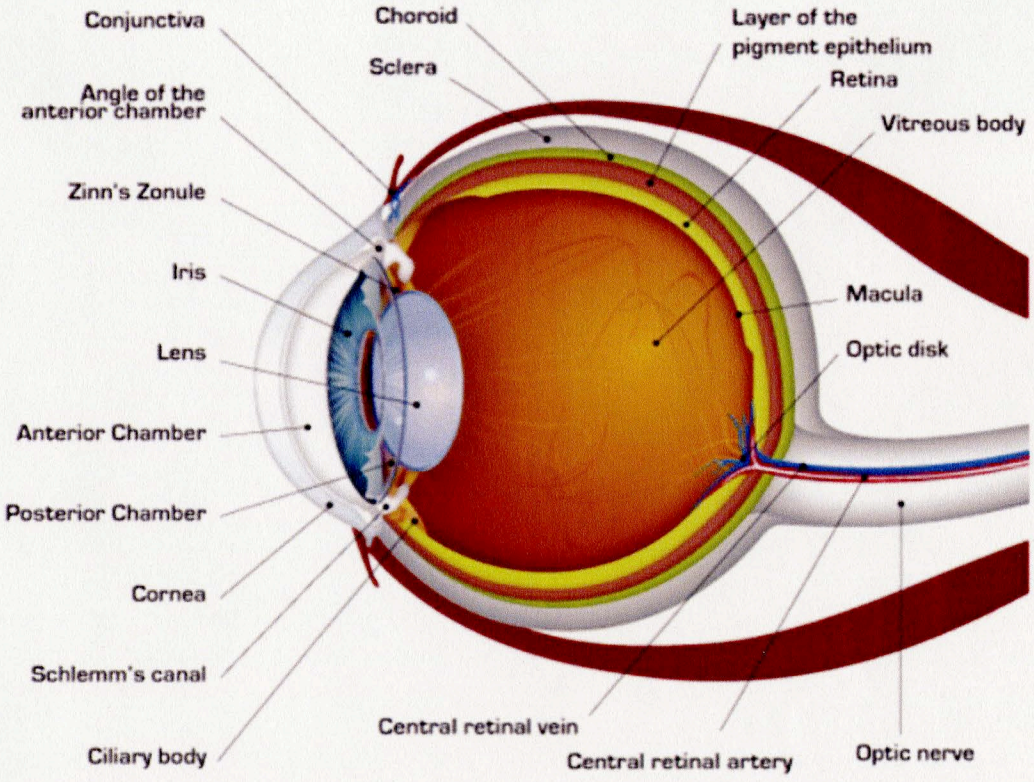

Figure A.1 Overall structure of the eye

A.2. Types of Glaucoma

There are several different types of glaucoma. Primary open angle glaucoma is the most common form type of glaucoma, affecting two-thirds glaucoma patients [Gupta]. The increased IOP in primary open angle glaucoma develops because of resistance to aqueous humor outflow through the normal drainage pathways, in particular the trabecular meshwork. In another type of glaucoma, normal tension glaucoma, the IOP is within the normal range, 10-20 mm Hg, but damage to the RGCs still occurs.

Possible causes of normal tension glaucoma include "poor blood flow to the optical disk, increased susceptibility to disk damage at lower IOP," or low cerebral spinal fluid pressure [Gupta, Ren]. The exact causes of normal tension glaucoma are still unknown.

Primary angle closure glaucoma is yet another form of glaucoma, characterized by "contact between the peripheral iris and posterior trabecular meshwork," with no other abnormalities in the eye [Amerasingh]. In angle closure glaucoma, the angle between the cornea and the iris essentially closes [GRF]. There are four different mechanisms implicated in the development of angle closure glaucoma including pupil-block, anterior non-pupil block, lens related and retrolenticular pathways [Amerasingh]. Secondary forms of both open angle (pigmentary or pseudoexfoliative) and angle closure glaucoma are the result of mechanical or physical blockage of the outflow pathways, or changes in its structure due to inflammation, eye injury, cataracts or diabetes. Congenital glaucoma occurs in infants when the aqueous humor outflow pathway is not formed properly in the womb. Other rare types of glaucoma include neovascular glaucoma and irido corneal endothelial syndrome [GRF]. Treatment regimes for glaucoma should account for the type that manifests, since different therapies are more effective for specific variations.

A.3. Prevalence of Glaucoma and Risk Factors

The distinct forms of glaucoma affect different populations at varying rates, which makes it difficult to obtain consistent numbers on the prevalence of the various forms of glaucoma. In the United States it is estimated that primary open angle glaucoma is the diagnosis in almost 90% of glaucoma cases [GRF]. Normal tension glaucoma is

more common in Japan, accounting for an estimated two-thirds of cases there [Gupta]. In India and East Asia primary angle closure glaucoma is more common than in other regions, affecting roughly half of those patients who have glaucoma in India and causing bilateral blindness in an astounding ninety percent of patients in China [Gupta, Amerasingh].

There are other risk factors for glaucoma, including family history, age, race, gender, diabetes, and high blood pressure [Gupta]. The prevalence of glaucoma increases with age, with visual impairment being largely confined to adults over 50. A genetic factor is present in some cases of glaucoma, so different genes are being studied for their role in the development in glaucoma. In particular, mutations in the myocilin (MYOC) trabecular meshwork inducible glucocorticoid response (TIGR) gene cause the onset of both juvenile and adult onset primary open angle glaucoma [Huang]. Race is a factor as well, with African Americans and Hispanics being roughly 4 times more likely to develop glaucoma than any other race [Vetrugno, Kim 2010]. Additionally, women are more likely to have chronic angle closure glaucoma and normal tension glaucoma than men. People who are far-sighted have shown an increased incidence of glaucoma. Angiopathy in Diabetes can lead to the progression of glaucoma, and high blood pressure can increase the IOP.

Appendix B: Glaucoma Medications

B.1. Beta Blockers

Beta blockers directly decrease aqueous humor production in the ciliary body [GRF]. There are several beta blockers currently on the market, including a few variations of timolol (i.e. timolol maleate and timolol hemihydrate), betaxolol, metipranolol, pindolol, carteolol, and levobunolol. These medications were first introduced in 1979 and have been the first line of therapy for glaucoma since, with timolol as the gold standard for glaucoma treatment [Vetrugno]. The usage of beta blockers has declined since 1992 from two-thirds of all glaucoma cases to only one-third of glaucoma cases, mainly due to their side effects. The price of these drugs is dependent on the exact formulation, but they range from costing patients $130 a year (generic timolol maleate 0.5%) to $370 a year (betaxolol hydrochloride) [Vold]. They are usually applied once or twice a day and the concentration, not the dosing regimen, is varied to modify the reduction in IOP.

Typically beta blockers are both safe and effective, but there can be some significant side effects associated with their use. The type and severity of the side effects depend on the selectivity of the beta blocker. For example, betaxolol has fewer side effects than timolol or levobunolol because it is selective to the beta-1 receptors, but it is not as effective in controlling IOP [Vetrugno]. Many patients say they experience stinging, burning, red eye, itching, tearing, and loss of feeling in the cornea when applying certain beta blockers [Gupta]. Non-selective beta blockers can make respiratory

symptoms in patients with asthma or bradycardia much worse, and can cause bronchospasm, headache, dizziness, or hypotension [Schwarzt, Gupta].

Cardiovascular function in patients can also be adversely affected through the systemic absorption of these medications since beta blockers decrease cardiac output [GRF]. "These agents should be used with caution in any patient with heart disease, heart block, or cardiac failure" [Vetrugno]. Systemic side effects of beta blockers can be minimized if patients close their eyes after application or use a technique called punctal occlusion, which prevents medication from entering systemic circulation through the tear drainage ducts [GRF]. In patients with diabetes, the symptoms of hypoglycemia may be hidden by the use of beta blockers. Long term effects associated with the use of beta blockers include mood alterations, hallucinations, memory loss, impotence, and loss of exercise tolerance.

The effectiveness of all the beta blockers in lowering IOP is similar, even though their structures differ. On average, timolol reduces IOP by 20-35% [Gupta]. Beta blockers such as carteolol and levobunolol tend to reduce side effects because they also mimic sympathetic nervous system activity. Carteolol showed a median decrease in IOP of 22-25% during clinical trials, and did not cause painful stinging or irritation in patients during application. A major issue with beta blockers is the fact that they are not as effective at night in lowering IOP and can cause nocturnal arterial hypotension, potentially leading to quicker visual field loss in susceptible individuals [Hayreh]. Overall, though beta blockers effectively lower IOP their use as a monotherapy for

glaucoma has decreased significantly in recent years due to their systemic and ocular side effects.

B.2. Prostaglandin Analogs

Prostaglandin analogs are thought to increase the outflow of ocular fluid through the less commonly used uveoscleral pathway, by altering the local permeability and pressure gradients [Gupta]. Due to their mechanism of action they tend to be more effective in patients with open angle glaucoma [GRF]. All the prostaglandins are partial agonists of the FP prostanoid receptor with the exception of travaprost, which is a full agonist. Several different medications on the market include travaprost, bimatoprost, and latanoprost. Latanoprost was introduced in 1996 as the first prostaglandin analog on the market, after which it eventually has become one of the most popular prescribed treatments for glaucoma.

Initially prostaglandins were not marketed for use on glaucoma patients because they caused inflammation, but after modification to the molecule the inflammation was reduced while the IOP lowering properties were maintained. The major benefit of using prostaglandins is that they have very few systemic side effects, mainly due to their rapid elimination half-life, though a few patients reported headaches, flu-like symptoms, and muscle pain [Vetrugno]. Prostaglandins do have ocular side effects, including changing pigmentation/darkening in the eye, growth of eyelashes, stinging, itching, burning, conjunctival hyperemia and blurred vision.

All of the prostaglandin analogs have similar safety profiles, and all of them are just as effective as beta blockers, lowering IOP about 20-35% from baseline [Vetrugno]. Prostaglandin analogs are effective at low concentrations, with the typical concentration of latanoprost and travaprost medications around 0.004%. At very high doses they can cause an increase in IOP, so care must be taken to ensure correct dosing. In addition to lowering IOP, some prostaglandin analogs have been found to increase microcirculation in the optic nerve head, which could potentially help protect the cells of the optic nerve from damage [Gupta]. A benefit of prostaglandin analogs over beta blockers is they do not become less effective at nighttime and are stable compounds that do not usually require refrigeration. Also, prostaglandins typically only require one application per day, which helps improve patient adherence. Due to their numerous positive qualities and minimal side effects, prostaglandin analogs are used to treat roughly 35% of glaucoma cases [Stein]. A possible issue with prostaglandins is that they cost slightly more than other treatments, with latanoprost averaging $350 per year for patients [Vold]. Prostaglandins are definitely a promising newer class of drugs for the treatment of glaucoma.

B.3. Adrenergic Agonists

Adrenergic agonists lower IOP by increasing aqueous humor outflow through both the trabecular meshwork and uveoscleral pathways [Gupta]. With the use of general adrenergic agonists, both the beta and alpha adrenoreceptors in the eye are stimulated. The main adrenergic agonists used for the treatment of glaucoma are epinephrine and

dipivefrin. Dipivefrin is a prodrug of epinephrine that does not require as high of a dosage because the addition of lipophilic functional groups allows it to more easily penetrate the cornea. Epinephrine lowers IOP by 15-25% while dipivefrin lowers IOP by 20-24%. Ocular side effects of using adrenergic agonists include blurred vision and conjunctival hyperemia, while systemic side infects include headaches, heart palpitations, high blood pressure, and anxiety [GFR]. The use of these epinephrine compounds has decreased in recent years, going from 16% of glaucoma cases in 1992 to only 0.6% in 2002 [Stein].

Alpha-2 adrenergic agonists used for the treatment of glaucoma include clonidine, apraclonidine and brimonidine. These medications decrease aqueous humor production while slightly increasing aqueous humor outflow. Alpha agonists are relatively selective to the alpha-2 adrenergic receptors, with some activity at the alpha-1 adrenergic receptors. Apraclonidine and brimonidine both have similar efficacies in lowering IOP, giving a 20-27% reduction in IOP [Gupta]. Also, brimonidine has a potential neuroprotective effect that could prevent deterioration of the retinal ganglion cells [Robin]. Though these medications sufficiently lower IOP, the necessity of two or three applications a day can adversely affect patient adherence.

The alpha-2 specific agonists overall have less side affects than the non-specific ones. Clonidine was the first alpha agonist used, but due to concerns of arterial hypotension and decreased blood flow to the optic disk more specific alpha agonists were developed [Vetrugno]. Brimonidine is around 30 times more selective for the alpha-2 receptor and is more lipophilic than the other alpha agonists, which increases its diffusion

through the cornea [Schuman]. Unlike clonidine, brimonidine cannot cross the blood-brain barrier and therefore has less systemic side effects. Burning and stinging of the eyes with application along with an allergic reaction leading to conjunctivitis are possible side effects of using alpha agonists [GRF].

In addition, some patients report fatigue, irritability, headaches, dry mouth, and dry nose with the use of alpha agonists. Alpha agonists, in particular apraclonidine, can become ineffective after prolonged use because of increased drug tolerance, or tachyphylaxis [Gupta]. For this reason, some alpha agonists are only indicated for short-term use. Alpha agonists were first introduced in 1996, and their use peaked in 2000 at treating 22% of glaucoma cases after which their use decreased to treating 17% of glaucoma cases by 2002. On average, brimonidine costs roughly $270 a year for patients [Vold]. Though alpha agonists are currently fairly common, their utilization is decreasing as more medications with fewer side effects are developed.

B.4. Carbonic Anhydrase Inhibitors

There are two types of carbonic anhydrase inhibitors (CAIs) that are utilized for the treatment of glaucoma: Topical CAIs and Systemic/Orally delivered CAIs. Carbonic anhydrase inhibitors function by decreasing aqueous humor production. The reaction converting water and carbon dioxide to a proton and bicarbonate is an essential step in the production of aqueous humor that is catalyzed by the carbonic anhydrase enzyme. The carbonic anhydrase enzyme's function is stopped by the use of the CAIs, thereby slowing aqueous humor production and lowering IOP. Commercially available drugs

include acetazolamide or methazolamide pills, and dorzolamide or brinzolamide eye drops [Gupta]. A 16-26% reduction in IOP is observed with dorzolamide, and CAIs may have the added benefit of increased ocular blood flow [Vetrugno]. The frequency of administration, three times a day for dorzolamide, may cause poor adherence in patients with CAIs as part of their regimen.

The systemic CAIs have many more adverse side effects than the topically delivered CAIs. Oral CAIs can cause fatigue, tingling in the extremities, weight loss, depression, upset stomach, aplastic anemia, and kidney stones. The side effects of topical CAIs are less severe and include things such as stinging and burning sensations in the eye. Some patients experience an allergic reaction to topical CAIs, which can induce conjunctivitis. Brinzolamide causes less ocular discomfort than dorzolamide because it is more lipophilic and can more easily cross the cornea [Vetrugno].

Oral CAIs were used to treat 9% of glaucoma cases in 1992, a number that decreased to 2% by 2002 because of the very severe systemic side effects [Stein]. Topical CAIs were first introduced in 1998, after which they peaked at treating 11% of cases in 1999, a number that decreased to 6% by 2002. Frequently topical CAIs are combined with beta blockers, a treatment used for 10% of glaucoma cases by 2002. Dorzolamide costs approximately $280 per year while brinzolamide costs around $240 per year [Vold]. Timolol combined with dorzolamide costs $470 per year, which is a barrier for its use in patients with less money. Systemic CAIs have a great deal of harmful side effects, but topical CAIs are both safe and effective medications for treating glaucoma.

B.5. Parasympathomimetic Agents/Cholinergics/Miotics

Miotics were introduced in 1870, and were the first class of medication used for the treatment of glaucoma [Dietlein]. Parasympathomimetic agents lower IOP by increasing aqueous humor drainage through the trabecular meshwork. The increased outflow is achieved when the anterior chamber angle is widened due to the contraction of the pupil and action of the ciliary muscle. Pilocarpine, aceclidine, carbachol, and acetylcholine are different cholinergic agents on the market. The reduction in IOP with the use of cholinergic agents is comparable to that obtained with the use of timolol. Due to the short duration that miotics remain effective they need to be administered four times per day, a number that greatly reduces patient compliance.

The negative impact of cholinergic agents caused their use to greatly decline in recent years, despite their low cost and high efficacy [Schwarzt]. Bronchospasm, cardiac irregularities, and intestinal cramps can result from the use of cholinergic agents. Locally, miotics can cause extreme pupil constriction, brow ache, myopic shift, inflammation of the iris, and increased risk of retinal detachment. Thickening of the conjunctiva, cataracts, abnormal increase in tear production, and stinging are other possible side effects associated with the use of miotics [Vetrugno]. The use of cholinergic agents sharply declined between 1992 and 2002, going from 28% of glaucoma cases in 1992 to only 4% of glaucoma cases in 2002 [Stein]. Miotics are the oldest form of glaucoma treatment and have been the mainstay for years, but they have recently fallen out of favor due to their numerous harmful ocular side effects.

B.6. Hyperosmotic Agents and Investigational Medications

Hyperosmotic agents are used to temporarily lower extremely high IOPs. The hyperosmotic agents work by reducing the overall fluid volume in the eye. An osmotic gradient is created between the ocular fluids and the blood, causing aqueous humor to drain from the anterior chamber [Kolker]. Oral glycerol, isosorbide, and intravenous mannitol are commercially available hyperosmotic agents. Glycerol can cause nausea and vomiting, while pulmonary edema and congestive heart failure are severe complications that can result from the use of hyperosmotic agents. In cases of acute glaucoma hyperosmotic agents are very effective.

Numerous new drugs are being investigated for their potential efficacy and safety in the treatment of glaucoma. An increased glutamate level has been implicated in the progression of glaucoma because it increases intracellular calcium, resulting in cell death [Gupta]. Novel treatments looking into glutamate inhibition are currently under consideration. NMDA antagonists could protect the RGCs by blocking increase in glutamate. Memantine and eliprodil are two NMDA antagonists currently being studied for their efficacy in preventing RGC destruction [Robert].

The ability of a patient to resist visual loss at higher IOPs largely depends on their specific immune response. Recent studies have looked into vaccines that could potentially protect the RGCs from future damage. R16 is a peptide obtained from the RGCs that could potentially be used to vaccinate the eyes against IOP-induced RGC loss and that has shown promise in animal models [Bakalash]. Erythropoietin, caspase inhibitors and iNOS-2 inhibitors are other potential glaucoma treatments [Gupta].

Mineralocorticosteroids such as fludrocortisone, and calcium antagonists such as nimodipine or nifedipine have also been proposed for glaucoma therapy [Dietlein].

Appendix C: Other Conventional Surgeries and Laser Surgeries

C.1. Trabeculotomy and Goniotomy

Another type of glaucoma surgery is a trabeculotomy. In a trabeculotomy an incision is made in the trabecular meshwork to increase aqueous humor outflow [Dietlein]. A portion of the trabecular meshwork is not removed during a trabeculotomy as it is during a trabeculectomy. Also, an ab interno trabeculotomy "avoids the creation of a subconjunctival bleb associated with traditional trabeculectomy" [Sloan]. In a non-randomized comparative study, the success rate (defined as a final IOP less than 21 mm Hg, 20% reduction in IOP after 3 months, and no need for additional glaucoma surgery) of trabeculotomy ab interno was 65% [Sloan]. Using an IOP below 21 mm Hg as the only measure of success, another smaller experiment showed a success rate for the ab interno trabeculotomy procedure of 91% at the 24 month follow-up. Patients still had to take medication following the ab interno trabeculotomy, though the average number of medications decreased from 3 to 1 on average. With all types of glaucoma surgery patients often need to continue using pharmaceutical agents in order to maintain a lower IOP.

Goniotomy is another type of glaucoma surgery during which the trabecular meshwork is surgically opened. During a goniotomy, the surgeon goes in through the cornea to make incisions along the anterior trabecular meshwork in a 90 to 120 degree

arc [Azuara-Blanco]. The main difference between a goniotomy and a trabeculotomy is that the trabecular meshwork is reached from inside of the anterior chamber during a goniotomy, but from outside of the anterior chamber during a trabeculotomy. Along with trabeculotomy, goniotomy is the preferred method for treating congenital glaucoma [Dietlein]. "Although successful in infant eyes with abnormally developed meshwork, goniotomy and trabeculotomy have been generally disappointing in adult eyes" [Johnson 2001]. Goniocurettage, similar to a goniotomy, is a promising newer procedure during which part of the trabecular meshwork is scraped away in a 90 degree arc with a curette.

C.2. Full Thickness Procedures

Several penetrating, full thickness procedures that can be used for the treatment of glaucoma are: an anterior lip sclerectomy, posterior lip sclerectomy, trephination, thermal sclerostomy, conventional sclerostomy, enzymatic sclerostomy, or iridenclesis [Nutan]. In a sclerectomy a full thickness portion of the sclera is entirely removed, usually with a punch, from the limbal portion of the sclera near to the cornea [Stamper 2009]. In the posterior lip sclerectomy, which is preferred by most surgeons over the anterior lip sclerectomy, the placement of the ab externo incision is slightly different than in the anterior approach and the tissue is removed from the back portion of the incision instead of the front portion of the incision.

Using an electrocautery tool in order to create an opening into the anterior chamber angle from the limbus is how a thermal sclerostomy is performed. The success rate of the thermal sclerostomy and the posterior lip sclerectomy was found to be the

same in a comparative study [Marion]. A trephination is where a fistula is created in the limbal area of the sclera near to the cornea using a trephine, which is a cylindrical surgical tool that can remove tissue sections [Stamper]. During an iridenclesis, part of the iris is pulled outwards from the anterior chamber and confined in the scleral sulcus incision. "The presumed mechanism was a 'wicking' of the aqueous by the iris tissue" [Stamper]. However, there are many complications with a iridenclesis, including chronic inflammation of the iris, infection, and sympathetic ophthalmia (inflammation of both eyes following trauma to one eye). Full thickness procedures are typically avoided due to their severe side effects.

Another type of glaucoma surgery called an iridotomy involves an incision of the iris to allow better aqueous humor outflow. Similar to an iridotomy, an iridectomy is a glaucoma filtration surgery during which a section of the iris is removed. The iridectomy procedure was first introduced in the 19th century, and is the oldest surgical treatment of glaucoma [Dietlein]. Iridotomy and iridectomy are typically used to treat patients suffering from angle closure glaucoma [Amerasingh]. In addition, a peripheral iridectomy is a routine part of many filtration procedures, including trabeculectomy. However, recent studies show that a peripheral iridectomy during a trabeculectomy should be avoided because it can cause more complications without any additional lowering of IOP [De Barros]. Though the laser version of an iridotomy is typically preferred because it is "less traumatic, associated with fewer complications, and can be performed on an outpatient basis," recent studies have shown that "small incision surgical iridectomy can be minimally invasive and effective" [Finger]. In certain cases of

glaucoma, a ciliarotomy or ciliectomy could be performed to either divide or remove sections of the ciliary body, thereby reducing the amount of aqueous humor production.

C.3. Laser Trabeculoplasty

Laser surgery is typically not as effective as conventional surgery in counteracting the elevated IOP seen in glaucoma patients, but it is much safer and does not have as many adverse side effects. One type of laser surgery commonly used is called laser trabeculoplasty. During this procedure a laser is directed at the angle of the eye to burn sections of the drainage channels and open them up to allow for better aqueous humor outflow [Mayo Clinic]. Some patients report a stinging sensation during laser treatment, so a topical anesthetic is typically used prior to surgery to reduce any discomfort [GRF]. Argon laser trabeculoplasty and selective laser trabeculoplasty are the two main types of laser therapy used for the treatment of glaucoma [GRF]. In argon laser trabeculoplasty a high energy laser is used to treat some of the filtration channels.

Selective laser trabeculoplasty (SLT) uses a low energy Nd:YAG laser to selectively treat certain areas of the outflow pathway while leaving the rest intact. Selective laser trabeculoplasty is very safe due to the low energy levels of the laser. Additionally, SLT has been shown to be just as effective as argon laser trabeculoplasty. If the IOP is not lowered sufficiently after the first treatment, the patient can undergo more laser treatments. Two sessions of laser trabeculoplasty are typically used. A 20% reduction in IOP is seen in 60% to 80% of patients who have undergone selective laser trabeculoplasty, though some studies show much lower success rates [Nagar, Dietlein].

Laser trabeculoplasty is typically only used for patients during the earlier stages of glaucoma because it only reduces IOP 5-6 mm Hg on average.

C.4. Laser Iridotomy and Laser Cyclophotocoagulation

A laser iridotomy is another surgical procedure that can be used to lower IOP [Mayo Clinic]. During this procedure a laser is used to create a hole in the iris and release pressure beneath the iris. This procedure was introduced in the 1970s, and it had a high failure rate of around 20% with a high closure rate of 30% when performed with an argon laser [De Silva]. The procedure has since undergone several modifications to improve efficacy and prevent serious complications such as hemorrhage of the iris. Today a Nd:YAG laser is used to perform this procedure, though an argon laser is sometimes also used in patients with darker eyes. Laser iridotomy is mainly used in patients with narrow or closed angles, and additional therapy is often necessary to control IOP for many patients with chronic open angle glaucoma. Complications of a laser iridotomy include corneal damage, cataract formation, hemorrhage, retinal burns, IOP spikes, or malignant glaucoma. An argon laser iridoplasty is another procedure which "involves the placement of a ring of contraction burns on the peripheral iris to contract the iris stroma near the angle, mechanically pulling open the angle" and lowering IOP [Amerasingh].

Laser cyclophotocoagulation is a procedure that uses a laser in order to destroy the epithelial cells of the ciliary body that produce aqueous humor [Dietlein]. The cells in the ciliary body are coagulated using a laser aimed from either inside the eye using an

endoscope or from outside the eye through the sclera, where no incision is necessary. Excessive treatment with this therapy can cause phthisis bulbi, or the shrinkage of the eye. Knowing the proper amount of treatment to apply during cyclophotocoagulation therapy is difficult, however, because a clear relationship between the amount of treatment and its effect on lowering IOP has not been established. The effectiveness of this procedure has yet to be concretely shown, and there is still controversy on the value of this treatment.

A recent retrospective study that reviewed cyclophotocoagulation showed that the treatment does have some promise and concluded that "diode laser cyclophotocoagulation is an efficient treatment for refractory glaucoma" [Iliev]. In the study, success (IOP between 6 mm Hg and 21 mm Hg) was seen in 69.5% of patients, failure or non-response was seen in 13% of patients, and hypotony was seen in 17.5% of patients. On average, the IOP went from 36.9 mm Hg before treatment to 15.3 mm Hg after treatment. Other studies have shown that severe vitreoretinal complications can occur following cyclophotocoagulation, including retinal detachment, choroidal neovascularization, endophthalmitis and vitreous haze [Wagenfeld]. More research needs to be conducted in order to be able to safely and effectively use cyclophotocoagulation as a glaucoma therapy since preliminary studies seem to indicate a high failure rate.

Appendix D: Traditional Glaucoma Drainage Devices

D.1. Molteno Implants

Molteno implants were introduced and 1969 as some of the first open-tube shunts for the treatment of glaucoma. Earlier in history setons, or solid stents that act like a wick, were used for the treatment of glaucoma and were made out of materials such as silk, cartilage, platinum, gold, or silicone [Stamper 2009]. The Molteno implants are made out of polypropylene and come in 135 mm^2 area single plate or 270 mm^2 area double plate configurations. A newer version of the Molteno implant, the Molteno 3 shunt, is both flexible and larger than the conventional design and can be either 175 mm^2 or 230 mm^2 in area [Shaarawy]. The shunt looks like a tube connected to a flat disk at one end. The tube enters the anterior chamber and bypasses the normal drainage pathways, shunting aqueous humor into a reservoir provided by the acrylic end plate.

Several studies on the potential advantages of using more plates in the Molteno implant have shown that more plates allow for greater aqueous filtration, but with a higher risk of postoperative hypotony [Molteno, Sherwood, Minckler]. One study showed that the double-plated Molteno implant had a success rate of 80% at one year, where success was defined as an IOP between 5 and 15 mm Hg [Taglia]. Another study on the use of the Molteno implant for neovascular glaucoma showed that it "provides useful long-term visual results in 39% of cases" [Every]. When compared with a trabeculectomy, patients with the Molteno implant had an average IOP of 15.4 mm Hg while patients with the trabeculectomy had an average IOP of 16.1 mm Hg at their 6 month follow-up, with different types of complications seen in both groups [Bluestein].

A variation of the Molteno implant called the Schocket procedure "involves shunting of aqueous via a tube to an encircling band" [Stamper 2009]. Though "results with the Schocket implant are similar to those with the Molteno implant,... most authors report slightly higher complication or reoperation rates" [Stamper 2009].

D.2. Baerveldt Implants

Baerveldt implants are made from silicone and come in two sizes, either 250 mm^2 or 350 mm^2. According to the designers and manufacturers of the valve, its "unique fenestrations allow the growth of fibrotic tissue through the fenestrations, thereby 'riveting' the bleb to the sclera" [Abbott]. Also, the device is "designed to control bleb height and volume" along with "minimizing the potential for ocular motility disturbances" [Abbott]. There is an optimum surface area for these shunts, determined through many different studies. In one study, a 500 mm^2 Baerveldt device was determined to be too large because it did not perform as well as a 350 mm^2 device, with a 5-year success rate of 66% compared with a 5-year success rate of 79% for the smaller device [Britt]. Another study showed very little difference between IOP reduction of the 250 mm^2 device and IOP reduction of the 350 mm^2 device at almost 3 years after implantation in people of Asian descent [Seah].

Based on these and other previous studies, an implant size between 250 mm^2 and 350 mm^2 seems to be the most effective compromise between fibrous encapsulation and overfiltration. Despite the potential long term benefits of using a larger implant, many surgeons prefer to use the smaller implants to allow for easier shunt insertion and to

avoid the risk of hypotony seen with the larger implants [Shaarawy]. The highest success rate shown for this valve was 96% at one year following surgery (where success was defined as an IOP less than 21 mmHg and an IOP more than 20% below baseline), with an average IOP of 12.4 mm Hg [Gedde].

D.3. Krupin Valve

The Krupin valve is a device made from a silicone elastomer. It is a 13 x 18 mm oval that is 184 mm^2 in area [Shaarawy]. "The end of the tube has a four-leaflet valve that eliminates the need for a stent or ligating suture during the early postoperative period" and prevents hypotony [Burchfield]. Though the valve is useful in preventing overfiltration, it is prone to malfunction and blockage. One study that required surgical revision following implant failure in 7% of patients concluded that the valve could be later modified to improve outflow in a relatively safe manner.

Another study looking at the long-term results of the Krupin valve showed a 3 year success rate of 66% and a 6 year success rate of 34%, where success was defined as an IOP between 5 and 22 mm Hg without additional glaucoma filtering surgery or devastating complications [Mastropasqua]. In the same study 54% of the patients experienced hypotony immediately after surgery, while 43% experienced external conjunctival bleb failure and 18% experienced blockage of the shunt by fibrovascular tissue in the long term. These results showed that "postoperative early hypotony, the growth of fibrovascular tissue extending to the open end of the anterior chamber and eternal scarring of the conjunctival bleb are the three most common causes of failure of

valve implants in filtering surgery" [Mastropasqua]. Another comparative study with more stringent IOP criteria showed the Krupin valve had a one year success rate (defined as an IOP between 5 mm Hg and 15 mm Hg) of only 39% [Taglia].

D.4. Ahmed Valve

An Ahmed valve is another glaucoma drainage device. It comes in a 184 mm^2 area single plate or a 364 mm^2 area double plate configuration. The designers of the valve state that the Ahmed valve "utilizes a specially designed, tapered trapezoidal chamber to create a Venturi effect to help aqueous flow through the device" and reduce valve friction, along with elastic membranes that can change shape to help regulate fluid flow consistently [New World Medical]. The Ahmed valve can be made from either polypropylene or silicone. Several were conducted in order to determine if the material made a difference in overall outcome. One study showed that both polypropylene and silicone had the same effect on pressure, but with less complications related to the polypropylene valve, while another study showed that silicone had a greater IOP-lowering effect [Shaarawy, Law].

It seems that the material that a shunt is made of can have an impact on its long-term efficacy, but more studies need to be done to elucidate the exact impact of the material on patient outcomes. When compared to a double-plate Molteno implant and the Krupin eye valve, "the Ahmed implant is less likely to create problems leading to reoperations or visual acuity loss than the Molteno or Krupin implants" [Taglia]. A recent study comparing the Ahmed valve to a trabeculectomy concluded that the 5-year

success rates (with success defined as an IOP less than 21 mm Hg and an IOP more than 15% below base line) of both procedures were not significantly different [Tran].

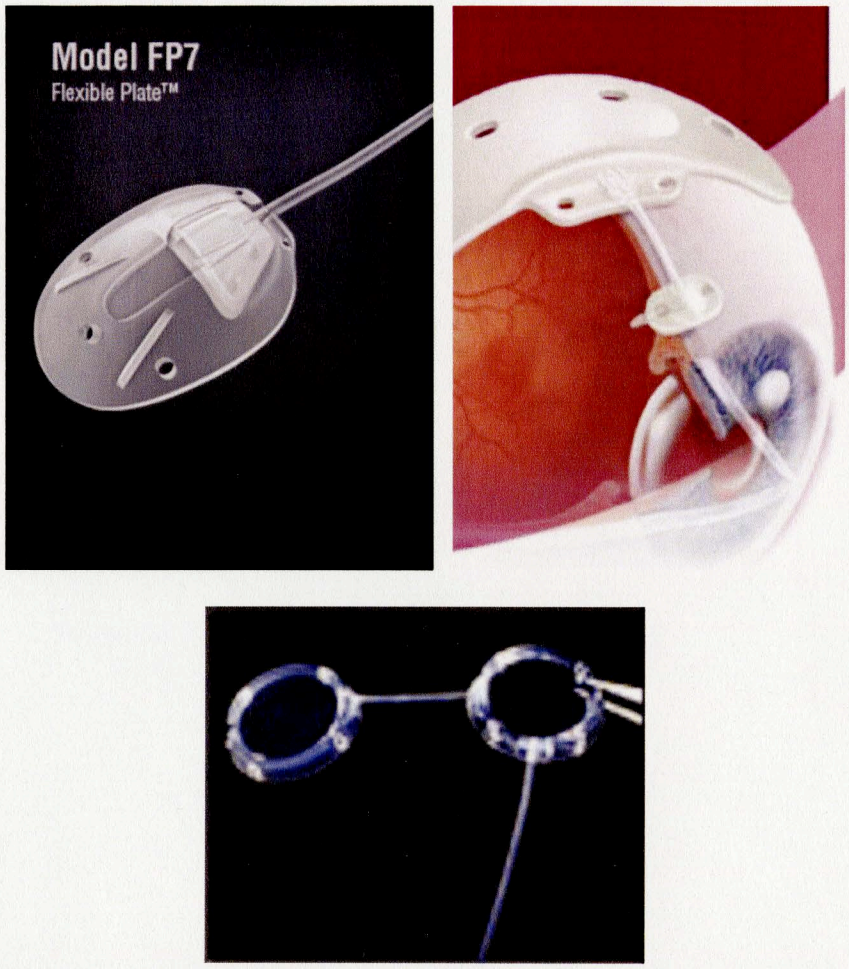

Figure D.1 Pictures of the Ahmed valve (top left), Baerveldt implant (top right), and Molteno implant (bottom) [New World Medical, Abbott, MedCompare]

D.5. Comparisons of Ahmed and Baerveldt Implants

There are a great deal of studies comparing the Ahmed valve and Baerveldt shunt, all showing varying outcomes. One study showed almost identical 4 year success rates (where success is defined as an IOP between 6 and 21 mm Hg with no additional glaucoma surgery needed and no devastating complications) of 62% in the Ahmed valve

group and 64% in the Baerveldt implant group, though patients with the Ahmed valve required more medication to control their IOP [Tsai 2006]. A different experiment concluded that "the Baerveldt-350 implant and the Ahmed valve had similar IOP control and surgical outcomes in patients with refractory glaucoma at 1-year follow-up," with a surgical success rate of 65.6% for both (where success is defined as an IOP between 6 and 22 mm Hg but also greater than 30% below baseline) [Syed].

The Baerveldt implant had a success rate (defined as an IOP of less than 22 mm Hg with no medication at the last follow-up visit) of 83.3% while the Ahmed valve had a success rate of 66.7% after 6 months in a smaller study on Asian patients [Wang]. Another more recent experiment also provided evidence that the "Ahmed S2 glaucoma valve may be less effective at lowering IOP than the Baerveldt 250-mm^2 Glaucoma Implant" [Goulet]. In this study, where success was defined as an IOP between 5 and 22 mm Hg with at least a 20% reduction in IOP, the Baerveldt group had a success rate of 85% while the Ahmed group had a success rate of 62% at the two year follow-up, along with a lower mean IOP and lower number of medications on average. Valved and non-valved devices seem to be equally effective in lowering IOP, but there are many factors that are difficult to account for during comparisons that affect the outcomes, including gender, race, number of previous glaucoma surgeries, type of glaucoma, and type of medications.

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